

MYCOSES OF MAN AND ANIMALS



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Foreword

At the time of his death in 1930 Dr Maurice Laperon had been engaged in preparing a second edition of his admirable *Précis de Mycologie*. The completion of this work then devolved on his friend and former pupil Professor R. Vanbreuseghem. In the new edition published in 1932 the work was divided into three parts. The first on General Mycology represented the greater part of the original book and preserved its outstanding characteristic. Descriptions of technical procedures were collected together and formed the second part. The third part devoted to Medical Mycology was an entirely new feature contributed by Professor Vanbreuseghem which greatly enhanced the value of the book. This part which has been translated into English by Dr J. W. Robinson is the subject of the present publication.

The translation has been made literally and the subject is presented to the English reader without addition to or alteration of the original text.

THE SCHOOL OF HYGIENE TROPICAL MEDICINE JACQUES VANBREUSEGHEM

Preface

IMMORTAL VAN DER LINDEN INTRODUCED TO THE SECOND
EDITION OF LANGERON *Tréc de Mycologie*

I am most grateful
to the author
Terry (Amst. 1910)

When in July 1940 Mlle Langeron and Dr Langeron finally entrusted me with the preparation of the second edition of the *Tréc de Mycologie* I accepted with hesitation for fear of detracting from the value of my master's work. I expected that at my disposal the manuscript to which Dr Langeron had told me he had devoted himself since the publication of the tenth edition of his *Tréc de Microscopie*. It proved however impossible to find the essential document. I had therefore to be content with what Mlle Langeron was kind enough to hand over to me: an interleaved copy of the first edition of the *Tréc de Mycologie* together with numerous offprints or microfilms collected with a view to the second edition. This material together with what I personally possessed formed the basis of the book I am now privileged to present.

The first edition comprised eleven chapters. I have divided them into three sections: General Mycology, Technique and Medical Mycology. General Mycology is based essentially on the first edition but the distribution of the material has been considerably modified. Additions have been made to it from Dr Langeron's notes and from my own reading. The second part devoted to technique follows the plan previously adopted but has been supplemented by new procedures which have appeared since 1940. For the third part Medical Mycology I take full responsibility. This topic was in the first edition limited to Chapter IX, entitled

What does medical mycology amount to? This title must be regarded as a appropriate means of rounding off the volume but at the same time it showed clearly Dr Langeron's intention to put the reader on guard against those who are hasty ready to create new mycoses on the strength of some mould isolated from a refractory case. Dr Langeron used to say to me: When people don't know which way to turn [which meant to me:] they think of fungi. I have completely replaced this ninth chapter by an entirely new third part in which the mycoses are studied in alphabetical order. It consists of seventeen chapters. I have endeavoured to omit nothing essential even of the most recent work and I have where ever possible set out what is known of animal mycology.

I feel that in this form the second edition would in no way run counter to the view of my late friend.

It may seem paradoxical to say that a scientific book owes more to many others than to its author. It is however true and particularly of the present volume which rests essentially on the work of Dr Langeron and on the experience of his long and fruitful career. Mme and Mlle Langeron have been extremely courteous in helping me gather the material necessary for this second edition and have greatly honoured me by entrusting me with the spiritual inheritance of one whom I knew well and so greatly admired.

Professor Edmond Sergent whom I met when he visited the Institut in Algiers kindly undertook the introduction of the present work to French readers: a more eminent ambassador could scarcely be imagined and I owe him a great debt of gratitude.

The Institute of Tropical Medicine at Antwerp has put at my disposal its precious laboratories and its remarkable library. For this I am indebted to its distinguished Director Professor Dubois to whom I tender my warmest thanks.

The interest shown by the Institute of Scientific Research in Central Africa (I.R.S.A.C.) in numerous branches of science led it to trust me with the carrying out of investigations in the medical mycology of the Belgian Congo and to subsidize my research for several years. I particularly owe my thanks to M. le Ministre De Bruyne, President of I.R.S.A.C., Prof. J. Rodhain, President of the Commission of Human and Animal Pathology, Prof. L. Van Den Berghe, Director of I.R.S.A.C. in Africa, Prof. J. J. Harroy, General Secretary of I.P.S.A.C. and all the members of the Administrative Council.

Professors Gerard and Penaux of the University of Brussels have given me their constant moral support and Prof. A. Dileq has given me much invaluable advice. I owe them my deepest gratitude.

My thanks are also due to Miss L. H. Georg (U.S.A.) and Dr M. F. (Brazil) for the fine microphotograph they have allowed me to use.

Many doctors and public health inspectors in the Belgian Congo have helped me considerably by sending material. I am particularly indebted to Drs. Borgers, Havit, Lejeune and Mathieu and to the Chief Inspector of Public Health M. Doorn. Mlle Van Hoof proved to be the most moderate of librarians and Mlle Van Rensel was a laboratory assistant and secretary, an indispensable daily help. My best thanks are due to all.

I am also infinitely grateful for the interest kindly shown in publishing this book and for their courtesy and attention always.

My wife who already had many claims on my affection has no required even more of it by helping me materially and morally through out the preparation of this work.

If I were allowed to dedicate the book it would be to the memory of two remarkable men of science who both at different stages contributed to the development of my scientific career: Professor André C. and

Let's work with Fleming, pioneer in the field of antibiotics and Dr. Maurice Langeron whose *Tree of Microbes* has rendered and continues to render the greatest service in laboratories all over the world.

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1994

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CHAPTER I

Mycoses caused by Actinomycetes

Introduction

At first glance it would seem improper to use the term "actinomycosis" for diseases produced by members of the Actinomycetaceae, but this is no longer tenable. In the first place, the designation actinomycosis traditionally refers to the localized cervicofacial actinomycosis, the disease is quite different from other mycoses caused by members of the Actinomycetaceae except for certain mycetomas produced by actinomycetes. Again, the actinomycetes have been so confused with one another that their precise taxonomy has only recently been clarified by the work of Waksman and Henric (1943). In this the Noctuidiae were considered to be distinct from local actinomycosis. Waksman's ignorance of certain fundamental characteristics of actinomycetaceae, whether aerobic or anaerobic, acid fast or not has invalidated much comparatively recent work. The separation of certain genera included in this chapter (erythraea, trichomyces) from the actinomycetes and Noctuidiae is considered to be justified not only on clinical grounds, but also because of lack of information as to the real nature of their usual agent. As it may well be made to refer to the erythraea, anti biotic properties of actinomycetes, for this subject the outstanding work of Welch (1947) should be consulted.

Broadly speaking, the actinomycetes are microorganisms possessing either rudimentary mycelium or well developed one not exceeding one micron in diameter. The former comprise the family Microbacteriaceae with but one genus *Microbacterium* as the causative bacteria. The latter with a true mycelium are nearer to fungi. They comprise two families, the Actinomycetaceae which reproduce by mycelial fragmentation and are divided into two genera *Actinomyces* and *Noctuidia* and the Streptomycetaceae which reproduce by conidia and comprise the two genera *Streptomyces* and *Micromonospora*. *Actinomyces* and *Noctuidia* will be dealt with in due course. The characters of the genera *Streptomyces* and *Micromonospora* may here be summarized to avoid repetition. These two genera are included in the family Streptomycetaceae by Waksman and Henric (1943) who distinguish them by the possession of vegetative mycelium which does not fragment into bacilli-form or cocciform elements and reproduce by means of spores.

Gen. *Streptomyces* Waksman and Henric 1943 *Streptomyces* species

are undoubtedly the most wide spread of all Actinomycete. According to Skinner, Frimmons and Tsuchiya (1948) they represent 90-95 per cent of the colonies obtained by spreading soil on agar but they are equally abundant in the atmosphere. Most of the work on Actinomycete is really concerned with *Streptomyces*. They are aerobic non acid fast form the mycelium of which fragments but little and which reproduces by conidia arranged in short chains. The conidia are frequently of polar form and the conidiophores (sporophore) are simple or branched. In the soil they convert nitrates to nitrites, break down proteins into simpler compounds and attack chitin and carbohydrates of a complex nature such as starch and cellulose. They are not pathogenic for man or animal but several species attack potato tubers (*Streptomyces* [*Actinomyces*] *scabiei*). The specific identification of *Streptomyces* is very difficult not only because the species characters have been poorly resolved but also because only a few species have been adequately described.

The formation of spores in short chains is one of the essential features of the genus. Unfortunately the appearance of these spores is not constant under all environmental conditions. Jones (1949) has shown recently that one fifth of the 1795 isolates made from different soil exhibited no constant morphology of the aerial mycelium and that after the first incubation 10 per cent of the isolates formed only vegetative mycelia, whence arises the possibility of confusion with the genus *Nocardia*.

Coret and Joubert (1951) have however isolated from a septarian actinomycetous of the domain an actinomycete with all the characters of *Streptomyces* (*S. griffithii*).

Genus *Micromonospora* Uchida 1931. *Micromonospora* includes aerobic and non acid resistant actinomycetes with reproductive spores borne either singly upon the aerial mycelium or aggregated in masses. This is a very little known genus of which all the species are apparently saprophytic and may be isolated from soil dung and decaying organic matter.

Wakeman and Henrici (1943) have tabulated the chief characteristics of the actinomycetes as follows—

ORDER ACTINOMYCETES

A Family *Mycobacteriaceae* Chester

Mycelium rudimentary or absent

Genus *Mycobacterium* Lehmann and Neumann

Acid fast or anisms

B Families *Actinomycetaceae* and *Streptomycetaceae*

Forming a true mycelium

I Family Actinomycetaceae Bauman

Vegetatively in long filamentous form or cocciform

(1) Genus *Actinomyces* Harkn 1877

Aerobic or microaerophilic parasites (or saprophytic) non-fast

(2) Genus *Nocardia* Thwaites 1899

Aerobic (ramipositive) fast or non-fast

II Family Streptomycesaceae Waksman and Henrici

Vegetatively in long filamentous form or cocciform

(1) Genus *Streptomyces* Waksman and Henrici 1941

Reproduction by conidia borne in small hair-like structures

(2) Genus *Micromonospora* Urey 1941

Reproduction by terminal spores borne singly or short paraphyses

A. ACTINOMYCOSIS

Definition

Actinomycosis is a chronic disease caused by *actinomyces* species characterized by a granuloma tissue which becomes and discharges through sinuses. The pus frequently includes sulphur yellow granules. The disease attacks man and cattle.

There appears to be general agreement on the designation of the disease but in the literature one occasionally comes across the name streptothricosis, nocardiosis or nocardiosis. This last term must be kept for disease caused by *Nocardia* species. The expression actinomycotic mycetoma has also been used by Langeron (1934) to designate all diseases caused by *actinomyces* species which give a clinical picture of mycetoma.

and to distinguish them from maduromycotic mycetoma caused by hyphomycetes. Jorgensen (1944) and also Jorgensen proposed the term Actinophytosis to designate all diseases in which one finds clubbed granules including the name of the microbe which causes them.

Historical

The first case of human actinomycosis appears to have been described in 1876 by Lebert since when in 1876 Bollinger described the disease in cattle. Harkn in 1877 called the pathogenesis a true *actinomyces* abscess relying upon the origin and appearance of the parasite within the tissues but he did not obtain cultures. In 1890 Bolstrom isolated an aerobic strain which he considered to be *actinomyces* Harkn but which was obviously not the organism responsible for the classical actinomycosis.

In 1891 Wolff and Israel isolated under anaerobic conditions of culture an *actinomyces* which Israel had previously isolated in 1844. Harkn in 1894 named this *actinomyces* strain I.

described by Urelov and bearing his name. This occurs as follows after fragmentation of the Actinomyces filaments the segments enlarge and repel one another by their end so as to assume an angular configuration. The fragmentation of the filaments is still very controversial and appears to be most clearly apparent in young colonies.

In trunks or exudates *Actinomyces israeli* takes the form either of branching Gram positive and non acid fast filaments or of sulphur yellow granules. These granules vary greatly in shape and dimensions. Frequently they are invisible to the naked eye but may sometimes be 1-2 mm in size. Their colour is traditionally described as sulphur yellow (sulphur granules of English and American writers) but many describe them as white or yellowish white. They are usually of soft consistence and may easily be crushed between cover slip and slide. Occasionally however hard and calcified granules are encountered. It is only too frequently stated that the colour of *Actinomyces israeli* are visible to the naked eye in general this is incorrect and a diagnosis of actinomycosis must not be rejected without a search for granules under the microscope. Moreover the presence of these granules is not absolutely necessary for diagnosis.

Actinomycosis granules freshly pressed out under the cover slip present on examination a polygonal or polycyclic appearance with in the centre a tangle of filaments which gradually open out towards the periphery where they present a what the French writers call *maïs* and the English clubs. These clubs are acid phobic. By using Gram staining method it can be demonstrated that the filament retain the centian violet whilst the modified Zuhl procedure shows that they are not acid fast.

The clubs which envelop the actinomycosis granules are not a constant feature and are not indispensable for diagnosis of the disease. Granules both with and without clubs may indeed be found in the urine. The origin of the clubs is still very debatable but they may well represent a reaction on the part of the organism utilized towards the pathogenic effect of the *Actinomyces israeli*. Certain workers however have apparently seen the clubs in culture.

It should be borne in mind that these clubs may be produced by more than one organism other than *Actinomyces israeli* (e.g. by *Streptococcus* and by *Actinobacillus lignieres* and also a great many other) they are not such a *Sporotrichum* *Phialophora* etc.

Symptomatology

Actinomycosis may be localized in many sites although the chief are cervicofacial, pulmonary and abdominal. Besides the cultural forms have been described which are really due to *Actinobacillus* and which should not be included within the strict boundaries of the actinomycosis. The purely cutaneous form, the ocular and the testicular form and the

Cervicofacial Actinomyces

This is the most frequent form and was found in 56.8 per cent of the series of cases studied by Cope (1939). The lesion usually first appears upon a swelling in the angle of the lower jaw. The skin over this swelling which soon becomes tough and woody is reddish violet in colour. The surface becomes irregular and soon exudes pus from several openings. In this pus occur the characteristic granules.

Often this is preceded by dental trouble such as extraction or a tonsillectomy.

The infection may extend to the pharynx and the orbit and there may be invasion of the salivary and lachrymal gland.

Diagnosis presents no difficulty.

Pulmonary Actinomyces

This is less frequent (— 3 per cent in Cope's series) and is seldom recognized before it has produced a fistular condition in the thoracic wall. In the absence of means of securing its true diagnosis it most nearly simulates tuberculosis. Signs of discharging pleurisy may be evident before the infection makes its external appearance but in most cases it is the development of a cutaneous sinus quickly followed by fistularization which permits of diagnosis.

In the pulmonary form it is well as in the abdominal form with which it may be confused there may be an emission of particles by way of the buccal cavity which enclose the *Actinomyces* mass. However both the pulmonary and the thoracic form may be derived one from the other and the pulmonary may frequently complicate the abdominal form.

Can *Actinomyces* be isolated from bronchial secretions in the absence of actinomycosis? This appears to have been confirmed in a recent and very interesting investigation by Hay (1949). This worker has searched systematically for *A. israel* in patients with bronchiectatic chronic abscesses and suppuration extending from the lung and has isolated it in 40 per cent of the cases. The secretions were first obtained by bronchoscopy or from operative fragment. In one quarter of the positive cases actinomycosis granules were found. It is acknowledged that the presence of *Actinomyces* without necessarily producing the disease complicates it and may lead to true actinomycosis with a characteristic development of sinuses.

Abdominal Actinomyces

This would appear to be caused by the disintegration of particles containing *Actinomyces israel* or to result from an extension of the pulmonary form.

This form (15 per cent of Cope's 1330 cases) is scarcely ever diagnosed before a laparotomy or an autopsy. The most frequent clinical indication is pain resembling that of appendicitis in the lower right region of the abdomen. Palpation reveals a soft lump in the ileocecal region. In the

absence of operation the infection may extend towards the muscle of the anterior abdominal wall and become fatal or it may get to the vertebral column attacking the vertebra and causing nervous troubles. A good many cases of *parametritis actinomycetosa* have been described and amongst the various explanations Stringer (1940) has pointed out the possibility of infection by an external route (repeated digital dilatation of the ann.)

Histopathology

For a specific histopathological diagnosis a tinomycetous granuloma must be shown to be present in the tissues. Without these the picture is insufficiently complete to permit of diagnostic conclusion. The exceptional presence of delicate solitary filament might incline the observer towards a diagnosis of actinomycosis. Stained with corn haematoxylin the granules would show a deeper central region and a reddish periphery since the clubs have a special affinity for eosin.

The granules make up the centre of a granulation tissue where white globules, giant cells and eosinophiles are found. Polymorphonuclear cells the degranulation of which lead according to certain workers to the formation of clubs are most directly in contact with the granule.

Following up previous deductions there may be found a corpusculum suppuratum or an internal abscess in either case in the absence of the typical granules a diagnosis of actinomycosis would not be warranted.

The use of Gram's staining procedure upon histological sections will differentiate any Gram positive filaments in the granule. If the granuloma should be caused by *Actinobacillus lignieres* the bacilliform elements within the granule are Gram negative. Should the granule be produced by a streptothrix the form of the latter is easily recognised.

Treatment

Although certain claims have been made for the treatment of tinomycosis by potassium iodide administered orally (3 drops of a saturated solution 3 times daily increased by 1 drop per day up to 30 drops 3 times daily) or sodium iodide intravenously (2 g. p. d.) progress has been remarkably modified by the introduction of sulphamide and penicillin.

Surgery is of some value in removing irreparable damage (gastrectomy, abdominal hysterectomy and bone resection) and X-ray in certain inflammatory conditions. Thus Lamberton (1943) for erysipeloid actinomycosis administered 140 r for two weeks divided up to a total dose of 1400-2000 r at a distance 30 cm with 4.6 mm aluminium filter, 1 min. minimum, 0 min. per filter for kilovoltage 120-140.

The sulphamide preferably sulphadiazine may be administered for 6 months maintaining a level of 10 mg per cent in the blood which the active dose is not well established being about rapid and short. In practice there is a tendency to combine penicillin and sulphamide.

Prognosis

The prognosis of actinomycosis is best in the purely cutaneous forms and good in the cervicofacial form. It is frankly bad in the pulmonary and abdominal forms.

On the whole what is known of the prognosis of actinomycosis has emerged from results obtained before the era of sulphamid and penicillin therapy and will doubtless be revised.

Differential Diagnosis

Only the cervicofacial form of actinomycosis presents a clinical picture which can be recognized. This is not to say, however, that its diagnosis must be resolved wholly on a clinical basis. It is to be expected that cases of such chronic evolution and polymorphic symptomatology as actinomycosis must inevitably be confused with the most varied diseases from which abscess complementing an abdominal form and simulating a hepatic metastasis pulmonary tuberculosis or cerebral tumour.

Mycological Diagnosis

This depends upon (i) the examination of actinomycosis granules in the pus and (ii) the isolation of the fungus in culture. The first pathological diagnosis is less reliable for actinomycosis than for other deeply seated mycoses for it may often be replaced by careful examination of the granules in the pus.

1 Examination of Granules in the Pus

The pus may be obtained from the sinus, diluted with physiological saline and examined in a petri dish placed against a dark background or slit red on guinea. As already mentioned granules are not always visible to the naked eye and they occasionally attain a size of 1 mm but never more than 2 mm. They should be carefully examined with a hand lens or whilst in the petri dish under the lamocul microscope.

Conant *et al* (1949) recommend the application on the surfaces of dry sterile gauze from the meshes of which granules may be obtained the moccasin after the application.

The granules are small masses of sterile white or yellowish soft and exceptionally crystalline.

2 Culture of Actinomycosis strains

Cultures of *A. israeli* are difficult to obtain because the parasite is anaerobic or microaerophilous and though it may be easily grown and isolated as an anaerobe in pure culture or from pathological products which only contain anaerobes the isolation is much more difficult from contaminated sources. Further *A. israeli* is a delicate organism and vaccination from the nutritional point of view. Lastly this parasite can only with difficulty be maintained in the laboratory it requires frequent sub culture and even so the cultures eventually die. Most workers seem unable to

maintain their strains for more than 3-4 months. However according to Rosebury (1944) if the strains die out in spite of persistent attempts to maintain them upon a given medium they may be perpetuated easily by transplantation upon different media as in a rotation.

Several media are suitable for the culture of *A. israeli* for example nutrient agar with the addition of 1 per cent glucose, Dorsett medium or glycerine egg medium nutrient broth and a medium which has given Rosebury the best results namely Difco's Bacto brain infusion added to 2 per cent agar. This culture medium which has the advantage of a relatively stable composition may also be improved in any laboratory.

A. israeli varies from strain to strain and from one day to another in its tolerance for oxygen but for satisfactory results it must always be cultured under conditions of partial or total anaerobiosis.

The easiest method of ensuring partial anaerobiosis is to introduce the material for culture into the bottom of a tube of nutrient broth or nutrient agar to which has been added 1 per cent glucose or Bacto brain infusion in 2 per cent agar kept at the bottom of the test tube liquid on the water bath and inoculated after cooling but before solidification of the agar (shake cultures). The actinomyces granules collected under aseptic conditions are possible and washed in several cubic centimetres of physiological saline is placed in a first tube and crushed against the side of the tube which is shaken to ensure the even distribution of the granule fragments in the bulk of the liquid agar. After this portion of the agar is transferred to a second tube and this is repeated to the extent of five or six tubes with the object of getting well isolated colonies.

The tubes are kept at 37°C and after 5 or 6 days are examined with a view to microscopical study of the colonies and further transplantation. The broth cultures lend themselves well for microscopical examination but on account of frequent contamination are of little use as a source of pure colonies. On the other hand agar cultures show well isolated colonies developed a little below the surface. Good examples have a particularly abundant accumulation of colonies 1 cm below the surface. By means of a Pasteur pipette a white colony is withdrawn having a surface film which is smeared out for staining by Gram's method for the recognition of interbranching Gram positive filaments or else bacilliiform elements exhibiting the angular growth of Cieslak. One or more colonies should be similarly withdrawn for transplantation.

If the inoculum is obviously very contaminated or if it is impossible to isolate the granules the procedure recommended by Rosebury (1944) is advised. The Bacto brain infusion added to 2 per cent agar is introduced into petri dishes then 1 ml of sterile broth is poured over the surface of the agar in each dish for 1 hour exposure. The material to be cultured is streaked onto the surface of the petri dishes with a

recovery of the platinum loop. The petri dishes are then placed at 37 C in a glass vessel enclosing 5 per cent CO₂ and hydrogen catalysed by warming over platinum or palladium. After 4 to 6 days those colonies are first removed which are white, firm and deeply set in the culture medium (type R) followed by the soft and friable ones (type S). According to Howbury the colonies are nearly all type R when first isolated and progressively changing to type S by subculturing. These occur at lower pH of the media.

Special care is needed with colonies which naturally anaerobic or microaerophilous have adapted themselves to free air and seem able to maintain themselves there for they are likely without warning to require return to non-aerated medium from time to time.

The optimum temperature for the development of *A. israel* is 37 C. At 22 C it will not grow. Warming for 10 minutes to 60-70 C will kill it. It will survive for 1 hour at 80 C. The optimum pH is 7-7.8.

Sugar is not fermented with gas production but the action of *A. israel* on several sugars (glucose, lactose, maltose, mannose, sucrose) results in acidification of the medium. The proteolytic action of the cultures, indole formation and nitro reduction to nitrites are variable. It is probably the same with haemolytic action so that the erection of new species based on these characters is clearly unjustifiable. Transfer of colonies should be carried out every 2 weeks but they can be kept alive for a month by refrigeration which is best carried out on dextrose agar.

Immunity Reaction

So far no known work carried out upon the cutaneous sensitivity of actinomycotic patient and the presence of antibodies in the blood has been performed with *A. israel* strain of *Actinomyces*. They have therefore no immunisation in relation to disease caused by *A. israel*.

Experimental Inoculation

Despite many attempts no one has yet succeeded in reproducing the characteristic lesions of actinomycosis by injecting *Actinomyces israelii* into animals.

Crootten (1934) by surgical introduction of fragments of 1% cation agar bearing colonies of *A. israel* into the abdomen of rabbit has noted the appearance of clubbed granules.

Emmons (1938) using strains of *A. bovis* (*israel*) of human or bovine origin failed completely. However by repeating inoculation intramuscularly in the 6 guinea pigs Emmons has been able to produce abscesses which lasted longer than in newly treated animals. From the pus he recovered actinomycotic granules devoid of club.

Howbury, Epps and Clark (1944) summarized their results as follows: repeated inoculation with pure cultures of 9 strains of *A. israel* animals injected into 4 guinea pigs and 18 rabbits have mostly yielded negative

results. However an experimental actinomycosis was obtained in animals, evolutive and mortal in guinea pigs and one rabbit localized and benign in other rabbits. Intravenous or intraperitoneal injections repeated with large amounts of *Actinomyces* appeared to be quite ineffective whereas single or repeated subcutaneous injections merely produced slow lesions from which the parasite could only rarely be isolated again. Fatal reactions were sometimes observed after intrapharyngeal injections or together with an inoculum of ground and sterilized calcareous calculus. Intracutaneous injections of living or sterile cultures in rabbits already inoculated and in not so treated revealed quite clearly that an allergic condition is associated with a progressive infection. Altogether the results obtained agree with the view that actinomycosis is an endogenous infection but show that the essential factor in the pathogenesis of this disease are as yet unknown.

It is well to remember that the method of acidification of sputum as used in connection with the inoculation of tubercular sputum in the guinea pig destroy *Actinomyces israeli*.

Actinomycosis in Animals

Actinomycosis is known in a great many animals both wild and domesticated. It has been noted especially frequently in cattle in certain regions. In the United States according to Skinner, Frimmons and Tschui (1919) actinomycosis is much more frequently found in abattoirs of the Middle West than in those of the South or the East but the difference thus attribute to the poorer systematic study of the disease in certain regions. The animals perhaps become contaminated with one another through the intermediate agency of fodder.

As already indicated only a proportion of the symptoms of actinomycosis in cattle can be attributed to *A. israeli* as many are in fact caused by *Actinobacillus lignieres*.

Baudou (1934) produced an excellent study of the paratuberculous actinomycosis in the dog better known as streptothricosis in dogs. The pathogenic agent is near to or identical with *A. israeli* and Baudou has suggested the name (*Ornithostreptothrix canis*) for it.

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The first case of nocardiosis was described in 1891 by Ippus, found and isolated an aerobic *Actinomyces* Cramp positive and seen in cerebral abscesses and meningeal exudates. Since then a number of cases (less than 100) have been described usually under name actinomycosis. Most cases were diagnosed at autopsy. The starting point is a pulmonary disease characterized by multiple abscesses. pulmonary lesions are frequently complicated by cerebral haemorrhagic subcutaneous abscesses. According to Kirby and McNaught out of 29 cases the lungs were involved in 29 and in 10 there was cerebral metastasis. The abscesses were deeply seated as in the patient of Binford and (1945) or they were situated in the ischio rectal fossa.

Prognosis is very poor and the case usually fatal (24 of the 29 cases Kirby and McNaught). Further therapeutic measures were lacking very recently however the introduction of sulphamides without having given any striking result undoubtedly on account of generally being used too late promise better for the future.

In pus and sputum *N. asteroides* composed of branching filaments simply bacilliform Cramp positive element. It does not form granules. The filaments and bacilliform components are equally acid fast as demonstrated by the use of a modified Ziehl-Neelsen technique. In it is useless to employ an acid alcohol solution to demonstrate this fast property for the filaments and bacilli decolorize. Cuthbert (1943) recommends that after staining with warm carbol fuchsin for five minutes destaining should be carried out with a 5 per cent sulphuric acid solution for a limited period the filamentous forms in sputum withstand the treatment not longer than 5 minutes while in culture the filamentous forms for 60 seconds and the bacilliform and cocciform element do not resist destaining beyond 5 minutes. All the same Drake and Henrici (1943) insist upon destaining with sulphuric acid alone to prevent also confusion of bacilliform element with Koch's bacillus is possible.

Nocardia asteroides may easily be cultured upon any medium suitable for aerobes at 37°C and at laboratory temperatures. Development however slow and several weeks are needed for the production of colonies. On agar the smooth colonies are oftentimes granular cerebriform and their colour progresses from yellow through orange to red. On Oxoid's medium where pigment develops most readily the colour is yellow or deep orange.

Experimental inoculation in the rabbit demonstrates the sensitivity to intravenous injection of *N. asteroides* the animal dies after a variable period usually rather short (1 day for Drake and Henrici [1943] 48 hours for Binford and Lam [1945] 29 days for Buchholz [1943] and Roux [1948]) exhibiting multiple abscesses of diameter 3 mm in organs. Subcutaneous or intramuscular injections in the rabbit require pig excreta in localized abscess formation at the inoculation site. The healing after suppuration. Peritoneal injection in the rabbit is possible without result whereas in the guinea pig it is fatal without treatment.

According to Constant and Rowbury (1948) *V. asteroide* is the only *Vaccinia* species pathogenic for laboratory animals.
 Drake and Henriksen (1949) prepared an asteroidin by evaporating broth cultures of *V. asteroide* killed by warming to one tenth of their volume. They have also studied in the rabbit and the guinea pig, the allergic reactions caused by intradermal inoculation of suspensions of *V. asteroide* in oil. After 30 days the animals showed a strong cutaneous reaction to an injection of crude unwarmed extract. The reaction was localized within the proteinaceous component of this extract. There is no cross reaction with Koch's cells.

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0 TRICHOMYCOSIS

Trichomy is or leprothy is a disease of the axillary hair characterized by yellow red or black nodules caused by *Vaccinia* or *V. asteroide* and various microorganisms.

The synonym of the disease is trichomycosis. Although known since Praxton (1894) first described it, it received the name of leprothy from Wilson while somewhat later Rich called it Trichomyxon palmellus. Yet other appellations are met with in the literature follow Trichomyxon axillaris Trichomyxon algens Trichomycetozoon Trichomyxon nodosum Trichomyxon aromaticum or Chromotrichomyxon.

Castellani (1911) first worked on the cause of trichomycosis and he recognized the three chief varieties yellow black and red and showed that the yellow variety is caused by an Actinomyces, the black and red varieties with microorganisms producing black and red pigment respectively. The two principal microorganisms are called leprothy and Micrococcus igneus Castellani (1911) and Micrococcus castellani.

Chalmers and O Farrel (1913) *Nocardia tenuis* Castellani 1911 has the following synonyms—

Trichomyces tenuis Castellani 1911

Cohni-treptothrix tenuis Ota 1924

Actinomyces tenuis Dodge 1931

Porcelli (1911) is credited with the rather difficult achievement of obtaining cultures of *N. tenuis*. It is now very clear that Macfie obtained cultures at Accra before the Italian worker by the following method: scrapings from infected hairs are soaked in absolute alcohol before being transferred to aseptic agar. After incubation for several days at 37°C a small transparent colony composed of fine and elongated radiating filaments appears on the surface of the agar. The colony grows slowly and remains translucent and almost invisible. Subculture upon a citrate agar grows more quickly—within 4 hours—but never any larger. Only the central regions of the colonies become opaque and resemble ground glass. The strain is easily maintained upon aseptic agar; all attempts to culture *N. tenuis* upon ordinary agar have failed. The non-acid fast filament is either Gram positive or negative in the young culture but becomes more strongly Gram positive in older ones.

Micrococcus nigrescens which accompanies *N. tenuis* in the black form of the disease has been cultured by Castellani (1911) whilst Chalmers and O Farrel in 1913 isolated *M. castellani*.

N. tenuis cannot be inoculated into human hair in situ.

Trichomyces shows a marked preference for attacking hair in the axillary regions but instances have been recorded of hair of the pubic and inguino-scrotal regions having been attacked (Chalmers and O Farrel). The black and red varieties are almost entirely tropical. However, Castellani and Wilkinson (1923) have described some cases of this disease in England. Different varieties may be encountered on the same person. The incubation period is unknown but is probably at least two weeks.

Hair attacked by *Leptothrix* exhibits either a dotted nodules or a thick and irregular sheath. Microscopical examination shows the nodules or sheaths to be composed of very fine filament often reduced to clubform or cocciform element which penetrate the hair cuticle and disengage themselves leaving the hair itself intact. According to Porcelli the hair is penetrated by *N. tenuis* but it may well be that this is a variation upon the study of poor preparation. In the black and red forms are found large black or red coccibodies the filament of *N. tenuis*.

Macfie (1916) discovered that besides the three principal forms there is a fourth brown form (*sar fusca*) which he described rather inadequately and from which he isolated a diplococcus associated with *N. tenuis*. Again Ping Ting Huang (1933) isolated from cases of *Leptothrix* in Japan an *Actinomyces sensu lato* with black cultures completely different from those of *N. tenuis*.

Those subject to trichomycosis often have axillary hyperhidrosis. It

and hardly even accompanied by an itch the erythrasma patches may when there is much loss of moisture produced by physical exercise or fever project and become slightly sore. They never develop vesicles. Erythrasma is mostly confined to the inguinal fold more rarely the axillary fold and occasionally the submammary fold. In man it is especially found in the lower part of the left inguinal fold contact with the scrotal skin apparently favouring its development. It is a disease of adults very common in men much less so in women and exceptional in children. Gougerot considers that erythrasma attack practically all men at some time or other. On the other hand American authorities consider the disease to be much less frequent in the United States than in Europe. It is not in evidence in the native Africans but it is very common amongst Europeans living in the tropics.

From the clinical point of view the disease is rather easy but erythrasma must be carefully distinguished from the epidermophytes especially *Hebra's eczema marginatum* and from *Pityriasis versicolor*. When the scales of the inguinal or axillary regions fail to show branching and relatively large segmented filaments characteristic of dermatophytes erythrasma may be borne in mind. The organism may be investigated by direct examination of the scales in chloral lactophenol or scales deposited in ether may be stained well in methylene blue and attached to a slide by colloidal dissolved in a solution comprising parts absolute alcohol to 1 part ether. Observation must be carried out under oil immersion objectives for the organism is too minute for dry objectives. It may present the appearance of short branching filaments of 1 μ maximum width or else of cocciform or bacilliform elements. Attempts to culture scrapings on Sabouraud's medium always give negative result.

Histologically erythrasma is characterized by slight hyperkeratosis and by minimum infiltration into the dermis.

Treatment of erythrasma is usually considered to be easy but we do not hold this opinion. Alleviation of symptoms is easily accomplished after the erythrasma has been irritated by perspiration and the friction accompanying the application of moist powders or 10 per cent boracic tincture. The use twice daily of 1 per cent iodized alcohol compared more favorably with a 20 per cent solution of sodium hypsulphite (chrysarobin) or resorcinol may bring about a dequamation and apyuric cure. But in most cases a more or less rapid relapse in the presumption of cure is usually premature for the patient will not stick to the treatment of erythrasma which can claim no novum.

CHAPTER II

Aspergillosis

THE AM *A. fumigatus* is not only a very common species of *Aspergillus* but also a very common and dangerous pathogen to man and animals. The most commonly isolated species is *A. fumigatus*. *Aspergillosis* is a well known disease of birds in captivity, especially aquatic species such as penguins. The question whether there is such a disease as human *aspergillosis* is a very controversial one, though very rare forms of the disease in human may exist.

The *A. fumigatus* comprises about 100 species, forms very widely distributed group of *Aspergillus* etc. which live in the soil and upon organic materials. Together with *A. niger* and *Mucor* species they are the most frequent contaminants of laboratories. Since spores are everywhere and are exposed to the atmosphere it is to be expected that *A. fumigatus* spores should appear upon them in abundance and if one were not wary of the danger of infection one might find it just as easy to establish a causality relation between a lesion which might appear and the fungus isolated from it. These highly controversial findings find too easy support in certain laboratory tests of lesions such as for example the greenish phase of certain onychomycosis attributed to *Aspergillus*. The correct conclusion is probably very different. *Aspergillus* are incapable of attacking a healthy nail but they are well able to grow in the breakdown product of nail attacked by a dermatophyte. The ease with which *A. fumigatus* species grow in culture and their inhibitory power on many organisms result in the isolation of a saprophytic *A. fumigatus* from the nail culture instead of the parasitic dermatophyte. This is no mere speculation for *Aspergillus* cannot attack keratin of our skin nutritive material for dermatophytes. Moreover, cases of so called mixed infections of nail by an *A. fumigatus* and a dermatophyte have been noted. Bertram (1946) reporting 13 cases of onychomycosis by *A. fumigatus* indicated that in one case the infection was caused by an *A. fumigatus* and *Trichophyton rubrum*. Without doubt the finger nail first attacked by *T. rubrum* were ultimately infected by the *A. fumigatus*. Another point in support of those who favour *aspergillosis* is that infection of *A. fumigatus* spores in laboratory animal often brings about a rapidly fatal condition. Even after eliminating those cases in which death of an animal is brought about by the infection of lungs alone, *Aspergillus* poses there still remains the undoubted pathogenicity for a large number of *Aspergillus* for laboratory animals.

In human pathology it is not very fit in the first place to just all

aspergillous diseases as eminently suspect and only to admit them with reluctance after all other possible causes have been eliminated. From the clinical uses of aspergillous in pigeon fanciers and wig makers it does not seem to be as convincing as formerly. In many pulmonary aspergillous diseases aspergilli and yeasts are secondary agents of infection to which are also often attributed a pathogenic power which they no longer possess (Conant *et al* (1944) p. 196) commenting upon the aspergillous pneumonia reported by Finch (1939) and also that of Jett (1942) concerning a cerebral abscess due to *Aspergillus* wrote: "In both cases mycelial filaments were present in the lesions and the diagnosis of aspergillous was extremely probable but it was not demonstrated."

If in man the diagnosis of aspergillous must be suspect in the majority of cases this is not so for animals and especially birds in captivity. These especially sea birds, as a very heavy toll and every year many specimens are lost from the world zoological gardens on account of aspergillous. Ainsworth and Rewell recently (1949) considered this question in an interesting article based upon their observations in the Zoological Gardens at Leventha Park, London. Aspergillous in birds is eminently a disease of the respiratory system. The air sacs, especially the anterior thoracic are the most frequently involved then come the lungs. In only three of the 8 cases described by Ainsworth and Rewell the abdominal viscera were attacked.

The lesions are nodular present in the air sacs or granuloma which invade the lungs. Aspergillous also can affect birds in captivity but in some cases death is so swift following capture of the bird that the pre-existence of the infection must be admitted. Opinion as to the etiology of aspergillous is divided between two groups, one maintaining that aspergillous is endemic among wild birds and that captivity turns it to a lethal disease, the other that aspergillous is a consequence of captivity to which certain particularly susceptible wild birds fall victim.

Whichever view be correct the birds cannot be treated once it is started and death soon supervenes. No effective therapeutic measures are known.

Identification of aspergillous species is difficult. Thum and Lajtha (1944) should be consulted on this subject.

Note: One must also exercise the greatest caution in accepting the results of microscopic analysis. *Penicillium*, *Mucor* and *Trichosporium* are respectively referred to as *Penicillium*, *Mucormycosis* and *Trichosporium* (cf. Conant *et al* 1944). The cultures are a wide proliferation and are frequent laboratory contaminants that grow on skin and are attached to their isolation in culture. The truth is that the fungus produces a clear specific and seldom at first a mycetozoa, whilst the label of the genus fungus has no proper symptomatic value.

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North American Blastomycosis or Gilchrist's Disease

Definition

North American blastomycosis or Gilchrist disease is a mycosis caused by *Blastomyces dermatitidis* Gilchrist and Stokes (1895) characterized by warty cutaneous lesions and lesions of more deeply seated organs with a predominance of pulmonary lesions.

The designation blastomycosis is somewhat unfortunate for it suggests the implication that the causative agent is a yeast since the fungus usually exhibits a yeast like morphology in certain stages of its development whether parasitic or cultural. Further it leads to confusion with so called European blastomycosis known also as cryptococcosis or torulosis caused by a true yeast *Torulopsis neoformans*. The eponym Gilchrist disease is preferable and pays tribute to the worker who in 1894 described the first known case.

It is noteworthy that in the United States the disease has often been referred to as Chicago disease (though this tendency is diminishing) because of the large number of cases originally from Chicago. The impression may sometimes have been gained that Chicago disease was of different origin than Gilchrist's disease but this is not actually the case.

Historical

In 1896 Gilchrist published his account of the case of dermatitis by a blastomycete which he had observed in 1894. In 1895 with Stokes he described a further case of this disease under the name of a case of common pseudo lupus and produced with his illustration a description of the parasite. The first description of this parasite must thus be attributed to these two authors whatever the name that it may ultimately receive and whatever the exact position to which it may eventually be assigned.

Importance and Geographical Distribution

Gilchrist disease is a mycotic dermatitis (of which some 300 cases have been described but only a hundred of these are unquestionable) due to *B. dermatitidis*. In 1939 Martin and Smith showed that of 34 published cases only 90 (about 26 per cent) were authentic instances of

Gilchrist disease. It is a disease of old age found especially in poor farming populations. Men are more frequently attacked than women in the proportion of about 10 to 1 (statistically given as 8 to 1 or 9 to 1). All races are susceptible, black race has sometimes been thought to be less sensitive than white but this is doubtful.

The geographical distribution of Gilchrist disease is strictly limited to the United States of America especially the Mississippi Valley. California and Illinois I already noted it frequent occurrence in the Chicago area has led to the name Chicago disease.

Apart from the United States certain cases have been recorded in England and Canada. Starr and Klotz (1949) in a recent analysis of reported for Canada could be established without doubt as Gilchrist disease and further their personal contribution of a new case is mentioned since no culture of the parasite could be made. In 1914 Brody reported an undoubted case (histopathology and culture) of North American blastomycosis which unexpectedly occurred upon an American soldier during his stay in France. The writer rejected the possibility of generally accepted incubation period (1 week to 4 months) as a man shorter than the time which had elapsed before the appearance of the symptoms (23 months) in that particular case. According to Brody there remained the possibility of contact infection from a patient sent from the United States.

Numerous cases of blastomycosis has been observed in many countries besides America. They are usually accompanied by mycological examination and seem appropriate to permit of classification and it seems appropriate to conclude that Gilchrist disease is almost entirely confined to the United States.

Ecology

There is yet no precise knowledge of the exact origin of the pathogenic fungus and although certain writers have considered themselves enabled to report that *B. dermatitidis* is widespread in nature this is difficult to prove. In 1914 Stober isolated from rotten wood found in the duck pond of one of his patients sufficient fungi to produce a mould similar to *B. dermatitidis* from which he prepared a vaccine to which a patient reacted in a greater interest are the observations of DeLamater (1948) that a sheep of 10 years old *B. dermatitidis* at various times from the urine of a patient with prostate trouble considered this urine may be a source of contamination of the soil. Following this up he inoculated sterilized soil with cultures of various strains of *B. dermatitidis* and obtained good growth of the mycelial form of the fungus. Unfortunately so far as we know the results obtained by this worker have been reported only in a very short note published on the occasion of a reunion one would wish for more detail.

Instances of contagion between man and man or dog and man have been noted but these are exceptional.

Pathogenic Agent

At the present time it seems unnecessary to attribute Ecthyma to any agent other than *Blastomyces dermatitidis* Gilchrist and Stokes 1899. However many attempts have been made to distinguish different species or varieties from amongst the isolated strains but it may well be that the various morphological forms are only variations of the same species. On the other hand although most authorities agree upon retaining for the fungus in question the name which was given by those who first studied it many synonyms are to be found in the literature of which the following are the commonest—

<i>Oidium dermatitidis</i> Rickett 1901	<i>Blastomyces ephelides</i> Dodge and Avers 1929
<i>Cryptococcus gilchristi</i> Vuillemin 1901	<i>Coccidium dermatitidis</i> Bagal 1931
<i>Symonema gilchristi</i> Burnburn and Cougerot 1909	<i>Microsporum</i> Line Agosti 1912
<i>Cryptococcus dermatitidis</i> Brumpt 1910	<i>Cleopora brevis</i> Castellan 1931
<i>Mycoderma dermatitidis</i> Brumpt 1910	<i>Blastomyces epulatus</i> Moore 1931
<i>Cleospore gemmella</i> Illici and Nannizzi 1927	<i>Blastomyces dermatitidis</i> Moore 1931
<i>Blastomyces dermatitidis</i> Castellani 1915	<i>Symonema dermatitidis</i> Illici 1931
<i>Blastomyces dermatitidis</i> Castellani 1915	<i>Cleopora dermatitidis</i> Redfern and Gifford 1934
	<i>Torula dermatitidis</i> Alvarado 1931
	<i>Symonema ephelides</i> Illici 1931

In tissue *Blastomyces dermatitidis* appears as a large rounded cell 4-15 μ and sometimes 3-7 μ in diameter with a rather thick wall which gives it the appearance of double contour (seen on the thick part of the budding). The bud which is usually smaller is cylindrical and a much finer membrane than the mother cell. Occasionally instead of forming a separate bud the cell elongates and the new form is absent of a hand mirror. More exceptionally a whole row of round cells is to be found arranged in a row before their separation from the mother. These cells can easily be distinguished from those of *Leishmania* the latter being much smaller and often stained with myxial fluid. The cell of *Torula neoformans* are surrounded by a gelatinous very thick within the tissue. The whole membrane of *Blastomyces dermatitidis* may be somewhat thicker than that of *Torula neoformans* the latter mycelium is most likely the commonest budding which characterizes this species should look for *Coccidium dermatitidis* and *Symonema* which are not found in the *Blastomyces dermatitidis*.

Cultures of *B. dermatitidis* are of two kind. On blood medium at 37 C the colonies are yellowish white, waxy of soft consistency and erumular in form. The colonies at 25 C or 30 C form a downy mould white at first but now and then brownish. Smooth colonies recovered from corems and others of floury appearance have been described.

The colonies cultured at 37 C represent the yeast like phase corresponding to that within the tissues. Here are to be found the large rounded and budding cells. The buds have a very large base which readily distinguishes them from true yeast. The outlines of mycelial filaments may also be found in this phase.

In colonies grown at 25 C or 30 C some yeast like elements may be found but the mycelial phase predominates. The mycelial filaments are not however so fully developed as in the first subcultures for the primary cultures represent an intermediate condition between the yeast like and the mycelial phases. In the first subcultures are found mycelial filaments which segment and branch and often constrict. Upon these mycelial filaments are borne the conidia either directly or by intermediary conidiophores 1-10 μ long. The conidia have smooth walls and their abundance and dimension vary considerably with the strain. They are round or lenticular with diameters 3-5 μ . In old cultures chlamydospores 7-18 μ may appear with thick and irregular walls.

Temperature is the essential factor which determines whether the yeast like or the mycelial phase shall appear. Raising the temperature up towards 37 C under laboratory conditions always gives the mycelial phase above 33 C from 30-37 C the yeast like phase is obtained and lowering this temperature results in the reappearance of the mycelial phase. One or the other of these phases may thus be obtained without difficulty and it is unnecessary to use complicated techniques or to pass again through an animal as is the case with *Histoplasma capsulatum* or *H. farciminosum*. However like *H. capsulatum* *B. dermatitidis* prefers solid media for its yeast like phase and it is only with extreme difficulty that it develops this phase in liquid media. The optimum pH is more on the acid side for the mycelial phase (pH 5-7) than for the yeast like phase (pH 6.5-8.5).

Levine and Ordal (1940) obtained excellent growth of *B. dermatitidis* upon glucose peptone media but they noted that the growth was slower if one used an inoculum made up of an mixture of spores from the mycelial phase than one made up from the yeastlike phase. Further on glucose media containing various salts and especially ammonium sulphate as the nitrogen source only the inoculum made up from the yeast like phase developed appreciably.

Symptomatology

Blastomyces dermatitidis probably has two means of access to humans. The one is cutaneous leading to a localized and somewhat benign symptomatology. The other is respiratory leading to the severe generalized form (in terms of blastomycosis of American authors) with lung damage in most

1948. The generalized form of blastomycosis can equally give rise to cutaneous lesions but as noted by Starrs and Klotz (1949) whereas in the primary cutaneous form the lesions are restricted to the exposed parts of the skin in the generalized form they are confined to the covered regions. Both forms will be dealt with in succession.

As already indicated the cutaneous lesions in the *primary cutaneous* form appear upon uncovered or exposed parts—face, hands, wrist, feet and ankle joint. The primary lesion is a pimple (papula) which slowly spreads at the periphery and becomes covered with a scab bearing a purulent pus. The edges of the spreading papula describe a perfect circle or are scurpiginous. The centre tends to heal in step with the spread of the lesion leaving an unpleasant scar. A typical feature of the margins is the enclosure of many small abscesses in which the parasite is easily detected. From these may be obtained material of the pure cutaneous form for purposes of culture. Wartiness is also typical of the lesion margins.

In the *generalized form* pulmonary lesions occur in 9 per cent of the cases, cutaneous lesions in 50 per cent, bone lesions in 60 per cent (especially ribs and vertebra), damage to liver, spleen and kidneys in 40 per cent and prostatic lesions in 70 per cent of patients. Lesions of the central nervous system occur in about 30 per cent of the cases.

Pulmonary lesions are usually the first sign of a generalized blastomycosis accompanied by rough, soreness, blood-streaked sputum and rather high fever. As the pulmonary symptom spreads the disease is disseminated to other organs notably with the formation of subcutaneous abscesses. These appear as nodules or gummy masses which often open and discharge. The margins of the ulcer thus formed are warty and enclose many small abscesses as in the primary cutaneous form.

Histopathology

Study of a cutaneous biopsy reveals two very special features. The first involves considerable epidermal hyperplasia which may cause the lesion to be taken for an epithelioma. The second is the presence of minute abscesses in the epidermal or dermal layers. In the epidermal case are found cells of *B. dermatitidis* liberated in the pus or enclosed in macrophages.

In other tissues the histological reaction is that of a granuloma leading to necrosis and suppuration. In the lungs are found a great many milium abscesses comprising cells of *B. dermatitidis*, multinucleated giant cells, plasma-cytes, epithelioid cells and occasional eosinophils.

Blastomycetum

Patient with North American blastomycosis are likely to manifest blastomycetum introduced intradermally by the formation of an erythematous papula which forms three or four days after the injection and may in very allergic patients be accompanied by a low abscess formation at the place of inoculation. When this reaction is positive it is to be concluded that

the patient has blastomycosis and is not of our ilk. A negative reaction does not however indicate freedom from blastomycosis and if it is known from another technique that the patient has Gilchrist disease the prognosis is not favorable. It would seem that the conclusions to be drawn from this procedure are similar to those which the leprosy specialists draw from Mitsuda's reaction in Hansen disease.

According to Jones and Martin (1941) the intracutaneous test technique is as follows: the cutaneous tests are carried out by a heat-killed vaccine prepared by suspending, in sterile physiological saline the yeast-like phase of a culture of *B. dermatitidis* on blood agar at 37°C. This suspension is centrifuged in a 15 ml. plasma tube and the sediment is once more suspended in enough physiological saline for a dilution of 1:1,000 by volume. The standard suspension is warmed for four hours at 60°C. Its sterility is confirmed by inoculating copiously upon a tube of blood agar which is maintained at 37°C for at least ten days. A 1% antiseptic (0.9 per cent trisresol) is added and 0.1 ml. of this suspension is injected intracutaneously to test the skin. During the following 15 or 20 minutes an erythematous zone usually appears at the level of the injection. This reaction has no specific value and occurs in patients with other types of pulmonary disease. The characteristic reaction commences 1-24 hours after injection and reaches a maximum in 4 days. It exactly resembles a positive tuberculin reaction. In very allergic patients a sterile abscess may appear at the point of inoculation. The appearance of a positive reaction may be considered as an indication of blastomycosis. This diagnosis is verified by the presence of antibodies in the patient's serum.

Demonstration of complement fixation is obtained by using antigen suspension from a living culture of the yeast-like phase obtained by growing on blood agar at 37°C. The titre of this suspension is determined experimentally. The proof of complement fixation in blastomycosis has peculiar value when it is positive. When negative it has no diagnostic significance but indicates in the case of a sufferer from Gilchrist disease a certain resistance of the organism. It is to be noted that this reaction is always negative in primary cutaneous form.

Result of these two reactions are obviously essential before considering therapeutic measures as their study plays an important role in treatment.

Treatment

Gilchrist disease is one of the rare systemic mycoses which can be cured. It is sensitive to potassium iodide which may therefore be applied in the most appropriate dose as a specific therapeutic measure.

Before administering potassium iodide it is necessary to find out the patient's sensitivity or more precisely his allergic condition. A cutaneous test is carried out by injection of killed vaccine already described. If the observed cutaneous reaction 4-48 hours after injection does not exceed 1 cm. in diameter potassium iodide may be administered by a rapid method scheduled by Comant *et al.* as follows: a saturated solution

of potassium iodide is administered at the rate of 3 times 3 drops per day and increased by 3 times 1 drop per day until the patient receives 3 times 100 drops per day. Having reached this dose a fresh beginning is made from 3 times 3 drops per day.

If the patient's reaction from intradermal injection of vaccine had a diameter greater than 1 cm the patient must be desensitized by progressive injections of killed vaccine before administering potassium iodide. This desensitization is carried out by subcutaneous injection every other day of 0.1, 0.3 to 1 ml of vaccine diluted to 1:100 if the reaction is 1 cm, 1:1000 if the reaction is 3 cm and 1:10,000 for a reaction exceeding 3 cm. Often after two weeks a sufficiently satisfactory desensitization is obtained to permit commencement of the potassium iodide treatment. In this case a slow method is employed consisting of administering a saturated solution of potassium iodide at the rate of 3 times 3 drops per day and increasing by 1 drop per day (not by 3 times 1 drop) until the dose 3 times 90 drops has been reached after which one reverts to the initial dose.

Vaccine injection may reveal a local or a general reaction according to the temperature rise. In these cases the commencement should be lower.

Iodine has also been introduced as sodium iodide injected intravenously (1 g. per day) or by the inhalation of ethyl iodide. In the cutaneous form it rarely often produces very useful results.

It is obvious that a symptomatic therapy will accompany a pathologic therapy.

Prognosis

Prognosis of North American blastomycosis differs according to whether the type in question is cutaneous or general. Whilst it is favourable in the former it is customarily fatal in 90 per cent (Martin and Smith) of cases of the generalized form. A already indicated a high antibody production is an unfavourable sign whilst a violent cutaneous reaction is the most hopeful. Death occurs from 2 to 3 years after the appearance of the first clinical signs.

Differential Diagnosis

It is of little value to enumerate the series of diseases with which blastomycosis may be confused in the generalized form. The cutaneous form may simulate warty tuberculosis, chromoblastomycosis, mercurial granuloma, syphilis and a great many other ulcerous conditions.

Mycological Diagnosis

The mycological diagnosis of blastomycosis is established by finding the parasite in the pus or in sections and by culture.

Animal inoculation as will presently be indicated is of little value.

Investigation of the parasite in the pus is made by first examining

without the addition of a clearing agent or possibly better after dilution of the pus with a drop of 20-30 per cent caustic potash. If budding forms are not found immediately it may prove useful to raise the cover slip and re-examine 4 hours later.

Sections of tissues may be stained by eosin haematoxylin but according to Dunn and Beeson (1930) superior results may be obtained by a silver impregnation method.

Culture may be initiated from pus, fluid from macroabscesses, sputum and urine. The suspected material is cultured upon blood agar and incubated at 37°C or on Sabouraud medium at laboratory temperature. Culture tubes must be retained for 3 weeks before being discarded.

Experimental Inoculation

As already mentioned it is difficult to inoculate blastomycosis in animals so this method is not recommended in establishing a diagnosis. It may be resorted to however to confirm the pathogenic nature and to retrieve a budding form of strain of *Blastomyces dermatitidis* already isolated in culture.

The most susceptible animal is the white mouse subjected to intraperitoneal inoculation. The guinea pig is relatively insensitive and the rabbit almost completely so. For successful inoculation it is necessary to use a large quantity of spores. In the white mouse it is possible to watch the appearance of granulations on the peritoneum from which budding forms of *B. dermatitidis* may be recovered.

Blastomycosis in Animals

Benbrook, Bryant and Symonds (1948) in a recent review of animal cases certified that 11 cases have been reported for the dog. In this animal the pulmonary lesion is dominant but H. Inzer has reported a case of cutaneous blastomycosis in a dog which infected two members of the family with which it lived. Benbrook et al. reported a case of blastomycosis in man; the diagnosis unfortunately was based only on histopathological examination.

Taxonomy

The characters of *Blastomyces dermatitidis* having been predicted it is perhaps better to refrain from setting forth a tentative systematic scheme for a parasite for which it is acknowledged by all that the binomial which designates it has no merit other than that of priority.

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CHAPTER IV

South American Blastomycosis or Lutz's Disease

Definition

This mycosis is characterised by granular masses in some of the lymphatic nodes, the membranes of the skin, the ganglia, the gastro-intestinal tract and the lungs. It is caused by *Blastomyces brasiliensis*. The nomenclature of this disease is rather ill established. North American workers naturally call it South American Blastomycosis in contrast with Coccidioidomycosis which is North American. Blastomycosis is South American a thoracic prefix the name Lutz's disease in honour of the worker who described the first cases. The eponym Lutz's Splendore-Almeida disease is also widely encountered in which Lutz's name is connected with that of Splendore, who was the first to cultivate the pathogenic fungus and also that of Almeida who made the first comprehensive study of the disease. Another name which is going out of fashion is that of paracoccidioidal granuloma an appellation given to a period when a parallel had been established between Lutz's disease and coccidioidomycosis.

Evidently the name Lutz's disease ought to be retained in preference to that of South American blastomycosis since the latter name gives undue prominence to the vestigial phrase which the parasite undergoes in the tissue.

Other synonyms are found in the literature particularly—

Brazilian blastomycosis	Paracoccidioidal granuloma
Malignant coccidioidal lymphogranuloma	Neotropical blastomycoidal granuloma
Coccidioidal lymphogranuloma	Paracoccidioidosis
Malignant paragoniary granuloma	Lutz mycosis
Blastomycosis etc. origin	

Historical

Lutz (1904) described the first two cases. He recognized the histology which distinguishes the causative agent of South American blastomycosis from those of coccidioidomycosis and Coccidioidomycosis respectively, but he considered the differences to be slight. Consequently he put the three diseases together under the common appellation American hypoblastomycosis.

Splendore (1909) described new cases of the disease and three years

later named the fungus responsible *Zygonema brasiliense*. Up to the present he has cultured it and inoculated it into several laboratory animals. Curiously enough since 1911 Splendore has observed perfectly the multiple budding typical of the parasite in the tissues a character regarded as specifically diagnostic by modern workers. In spite of this during the ensuing years the question remained unresolved and Lutz disease is regularly confused with coccidioidomycosis. It was not until 1937 that comparative study by Souza Campos and Almeida of the fungus responsible for the two diseases permitted them to be distinguished one from another. A little later Almeida proposed the genus *Paracoccidioides* in which he placed the agent of Lutz disease under the binomial *Paracoccidioides brasiliensis*.

In 1940 Conant and Howell established a correct parallelism between the agents of Culebrist and Lutz's diseases respectively they gave the name *Blastomyces brasiliensis* to the agent of South American blastomycosis.

Importance and Geographical Distribution

Lutz disease is a relatively frequent mycosis. Almeida has called attention to 500 cases in South America. According to Vergani and Bigliolo (1948) a thousand cases have been reported from Brazil.

The geographical distribution of the disease is essentially South American and especially Brazilian. In Argentina a good many cases have been reported some have been recognized in Paraguay, Peru and Uruguay. Apart from South America some rare cases have been discovered in Costa Rica.

The disease is found especially in young adult aged 30-40 with an absolutely great predominance of men to women (about 9 to 1).

All races are susceptible and the disease takes a heavy toll of manual workers in fields and on farms.

Etiology

Nothing is known of the origin of Lutz disease except that it seems to have a preference for land workers.

Pathogenic Agent

There appears to be only one pathogenic agent of this disease though several species have been described. The causal agent *Blastomyces brasiliensis* (Splendore 1911) Conant and Howell 1940. It is known under many synonyms especially—

<i>Zygonema brasiliense</i>	Splendore	<i>Paracoccidioides coccidiiformis</i>	
1911		Moore 1933	
<i>Paracoccidioides brasiliensis</i>		<i>Paracoccidioides</i>	Almeida 1937
			Almeida 1937

In tissue the organism takes the form of rounded, thick walled double contour 10-15 μ in diameter which reproduces by multiple budding.

boiling. All agree that the form with multiple budding, which may be somewhat difficult to demonstrate in tissues, is the only form diagnostic of the species. South American workers have dubbed it the wagon wheel (a *rueda d'automóvil*) or aeroplane motor (*motor d'aeroplano*) form.

Björk (1914) has published well documented work upon forms of *B. brasiliensis* in tissue upon budding. The rounded cells in tissues are either free or enclosed within giant cells. Budding may commence in larger cells up to 40μ in diameter. The buds, single or multiple are formidable size with diameter $0.5-1 \mu$ according to the size of the cell from which they are derived.

According to Björk, I agree that there also has been studied the matter the effect of tissue budding, and not a passing of the exterior of pores formed within the cell (cryptopores) of (C. fern and Pedicelli or c. c. c. sporulation of F. c. c.). In certain mother cell Björk has observed chromatin masses disposed round the periphery of the cell where they had been pushed by the formation of a central acute penetrating, not pocket formed by a thinning of the wall of the mother cell and going on to the bud after having been surrounded by little cytoplasm. There is no formation of little cells such as has been claimed to be due to the liberation of buds formed inside the cell.

(As with multiple buds are not encountered with equal frequency in tissues. They are most readily detected in tissues with no outlet per aquatum droppal fluid in tissues fluid and from tissues per tick scarcely or contain a.)

Cultures of *Blastomyces brasiliensis* which grow in places according to the incubation temperature. Whereas at laboratory temperature filamentous colonies are obtained in 17°C the colonies are made up of yeast like elements similar to those recovered from tissues. (Note: C. c. c. and H. (1914) these facts emphasize the similarity between *B. brasiliensis* and *B. dermatitidis*.)

The yeast like phase is also found in 17°C on the usual media addition of blood to the culture medium not being necessary. The colonies are glassy, cerebriform and strongly resemble yeast colonies. As already stated they contain elements of identical morphology with those of cell present in tissues. The buds are either single or multiple.

The filamentous phase grows slowly (sometimes four weeks are needed for primary culture) at laboratory temperature or at 37°C. The colonies are unusual, membranous or corrugated and cover the center with whitish down which tends to brown upon drying. The branched and segmented filament produced oval or round conidia similar to those found in colonies of *B. dermatitidis*. However the conidial forms of *P. dermatitidis* are less regular than those of *B. brasiliensis*.

Transition from one phase to the other is easily brought about by making a fresh inoculation from colonies of any phase whatever and utilizing the temperature necessary for the phase required.

Symptomatology

Intestinal disease takes two forms—localized and generalized or mixed.

1. In the localized type the lesions are cutaneo-mucosal lymphangitic or visceral. The cutaneo-mucosal lesions are localized in the buccal mucosa (tongue, cheek, pharynx, larynx, palate) and extend toward the exterior, encroaching upon the skin of the face. The lesions of the buccal cavity commence with a small papule which ulcerate and spread from the margins. The regional lymph nodes are rapidly involved. The cutaneous lesions are warty, papillomatous and ulcerate at the middle with marginal hypertrophy. The localized ganglionic lesions encroach upon the lymph nodes of the neck and produce a clinical picture strongly reminiscent of Hodgkin's disease. The lymph node may necrose and drain to the exterior through sinuses. The visceral localization is most frequently met with in the cecal and appendicular region.

In the mixed or generalized type are encountered bucco-lymphangitic, bucco-cutaneo-lymphangitic and cutaneo-lymphangitic symptoms associated with crippling of the gastro-intestinal tract and accompanied by hepatomegaly, splenomegaly, ascites. According to Verani (1918) more than 50 per cent of cases may be fatal. Lesions involving destruction of the epiphyses and the metaphyses. The frequency of pulmonary lesion in the mixed forms is still very controversial. The São Paulo school considers this type of localization to be rare but this view is obviously being modified if reliance is placed upon the report of the 1941 Conference on Intestinal Disease. The Rio de Janeiro authorities think on the contrary that lung involvement is very frequent and occurs in as many as 50 per cent of the cases. Fialho (1940) found pulmonary lesions in 84 per cent of the cases and in another series of 10 cases obtained them in 16 instances (94 per cent). Nave (Argentine) noted lung attack as inevitable. A ray-necrotic particular and mediastinal necrosis. Sputum is purulent and blood-streaked. Ritter has recently (1949) reported two cases of tumour, one cerebral the other cerebral, caused by *B. brahii*. But apparently the organism was not cultured.

In the localized form the general condition is well maintained while in the localization of lesions (bucco-pharyngeal form) there is a marked diminution of nutriment. In the generalized form the general condition is reaching a peak in the advanced and often fatal stage. There is no skeletal emaciation and no hypochromatous anaemia.

Histopathology

Histologically the lesions are entirely similar to those met with in Calchiet's disease and are characterized by the presence of giant multinucleated cells from which the pathogenic fungi are recovered in great numbers. There is an infiltration of the tissue by histiocytes, polymorphs and lymphocytes. Polynuclear neotrophs and eosinophils are more

rarely found. At the same time, the frequency of dysenteric colitis are more abundant.

Treatment

Most progress has resulted from the introduction of sulphonamide therapy by the South American workers. In fact only the sulphonamides are effective. It is not yet proved that their use results in final cure but it is quite certain that they prevent the spread of the disease from the primary focus and check the development of the secondary focus especially the pulmonary lesions.

Sulphapyridine may be administered as an initial dose of 100 mg/kg followed by a dose of 1 g repeated every four hours so long as the drug may be taken satisfactorily.

Patilha Gonçalves estimates that the blood concentration of sulphamerazine and sulphadiazine must be set at over 10 mg. per cent whilst that of sulphathiazole may be slightly less.

Luna Cast and Abbott (1947) report having cured a patient suffering from a fulminating pulmonary form by the administration of sulphamerazine at the rate of 3 g during the first day then 2 g up to a total dose of 37 g.

Potassium iodide has also been much used especially by Almeida. Its therapeutic action is however slow and therefore appears to be residual in disseminated cases if it is used.

As to vaccines too few results are yet available for any appraisal of their value.

Prognosis

In general up to the introduction of sulphonamide therapy prognosis has hitherto been regarded as fatal after an interval which does not appear to have been definitely ascertained.

In spite of the very favourable opinions of those who have used the sulphonamides in Lutz disease it still remains to be established that they effect a cure rather than that they simply arrest the disease.

Differential Diagnosis

There are various possible diagnoses according to the form which the disease may assume. The lymphangitic form may often be confused with Hodgkin disease. Pulmonary lesions may be taken for tuberculous, tumorous lesions for syphilis or South American kishimunias. The haemorrhages from intestinal lesions may be confused with the haemorrhages of other diseases in which blood is present in the stool.

There may be confusion with other mycoses such as histoplasmosis (histoplasmosis) and coccidioidomycosis.

Mycological Diagnosis

Specific diagnosis may be established in three ways:

1. *From isolation of the Organism* - The fungus responsible for Lutz

discrete may be looked for in secretion, abscess, sinus and putum. Pathological material obtained from a swab or by tapping with syringe may be smeared out on a slide in a small quantity of 10 to 20 per cent (30 per cent) and examined under a cover slip. It will be recalled that the yeast like elements which have a diameter as much as 60μ may produce one or more buds from $2-10 \mu$. Only the presence of the multiple budding forms confirms the diagnosis of Lutz disease since budding forms with single buds are also found in Gilchrist disease. Non budding forms will be distinguished from *Coccidioides immitis* by the presence of endospores in the latter organism. Certain forms of *C. immitis* however do not form endospores.

The pathogen may also be demonstrated in sections stained with counterstain, toluidine blue, Gram's or by one of the silver impregnation methods.

Culture Methods. Secretions, pus or biopsy specimens are cultured on Giboulaud's medium and kept at 25°C or 37°C . The culture tube must be returned for at least four weeks as the fungus grows very slowly in the primary cultures.

3. Animal Inoculation. This should be carried out by one of the techniques indicated under experimental inoculation.

In conclusion besides a diagnosis of *Blastomyces brasiliensis* may follow there must be evidence of form with multiple budding in the pathological product in culture kept at 25°C and in the tissue of inoculated animal.

Immunity

There is no specific reaction in Lutz disease. Complement fixation studies starting with cultural filtrates as antigen have given positive reactions not only for Lutz's disease but also for paratuberculosis, chromoblastomycosis and with the serum of patient afflicted with keratopharyngomycosis (Almeida).

On the other hand the intradermal injection of anti-culture filtrate from culture is followed by welling, erythema and occasionally discomfort. It is not certain whether this reaction is specific. The intradermal injection of coccidioidin produces no reaction but blastomycin (distillate from cultures of *B. dermatitidis*) may cause a positive reaction.

Neve de Silva has recently prepared an antiserum by exposing pus obtained from guinea pig testicle inoculated with *B. brasiliensis* for one hour at 30°C upon three consecutive days (tyndallization). Before the tyndallization process the pus which is rich in budding, is filtered fifteen times with physiological saline. Intradermal injection of 0.1 ml of this suspension produces in patient with Lutz disease a local inflammatory papule which lasts three days and tends to increase in certain cases. The reaction is negative in controls (control with other mycetes).

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Experimental Inoculation

Inoculations may be made either with pathological product or with cultures but are usually carried out with the latter. Unfortunately most workers fail to indicate whether they have used the yeast like or the filamentous phase both forms are pathogenic.

Both *in vivo* and *in vitro* have been used satisfactorily.

1. In the guinea pig, inoculation must be carried out in the testicle (cultured element are recovered 48 hours after inoculation then they are removed and return in tissue form by the sixth day. Clinically the lesion appears at first as a nodule accompanied exceptionally (4 times out of 4 guinea pigs inoculated according to Kialho and Gonçalves) by involvement of the regional lymph nodes which however always heal though it may be possible to recover the parasite from them. The orchitic stage is followed by a softening phase which is complete within a month of inoculation by this time the testicle is transformed to a mass of pus in which are present large numbers of budding forms. According to most observers however forms exhibiting multiple budding are here found but rarely.

2. In man intraperitoneal injection is necessary. Infection progresses slowly and 5 or 6 weeks after inoculation there may be detected small nodules containing budding forms in the mesentery and other organs. Neither of the animal species has shown generalized infection.

Taxonomy

The genus *Paracoccidioides* has been erected on account of the alleged resemblance between the pathogenic agent of coccidioidomycosis in man (11) and that of *Lutzia* (12). In fact the presence of endospores in the tissue forms of *C. immitis* and the existence of this fungus in unique cultural form conclusively separates it from *Blastoschizum brasiliensis*. On the contrary *B. brasiliensis* has strong affinities with *B. dermatitidis* like the latter it reproduces in the tissues by budding, is devoid of endospores and has its phases in culture depending upon temperature. The only distinguishing feature is the occurrence of multiple budding in the tissue form or the yeast like phase in cultures of *B. brasiliensis*.

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Apert from their differences as pathogens these three species may be distinguished from one another as follows: building, single in *B. derm.* (but single or double in *B. lob.*) and single or multipl. in *B. trax.*

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CHAPTER V

The Cephalosporioses

UNDER THIS NAME are included diseases produced by imperfect fungi of the genus *Cephalosporium* Corda, of which the type species is *Cephalosporium acremonium* Corda 1839. This saprophytic species (from soil decomposing plant remains dead in soil) has repeatedly been isolated from different types of lesions, and it is doubtful whether it is really pathogenic or not for man. A few number of *Cephalosporium* species are pathogenic for plants and certain arthropods.

The genus *Cephalosporium* typically has a vegetative mycelium made up of segmented hyphae which bear sporophores inserted at right angles upon which develop a mass of terminal spores of cylindrical in shape bound together by mucilage. The conidia are the microspores. The genus is distinguished from *Acromonium* Link 1809 in which the sporophores usually bear at their extremities one fixed rim spore which is easily detached.

Cephalosporium species have been isolated from epidermal lesions inflammatory or otherwise (intertrigo, onychia, vulvovaginitis) from mucous membranes (buccal, ocular, vesical) from blood and putum.

The multiplication of lesions together with the ubiquity of *Cephalosporium* species renders very suspect the already numerous work which attribute the production of a variety of lesions to various species of *Cephalosporium*. The well documented work of Coutin (1941) and Biquet (Les *Cephalosporioses* humaines. 4. *Parasitisme* (1948) pp. 36-384-99) may profitably be consulted on this matter.

CHAPTER VI

Chromoblastomycosis

Definition

Chromoblastomycosis is a chronic dermatosis caused by the accidental injection of various species of Fungi Imperfecti belonging to the genus *Phialophora*. Generally confined to one of the lower limbs it is characterized by warty nodules which develop slowly into a vast papillomatous sheet and end with or without ulceration. This infectious disease is not contagious.

The disease was first described under the name *errucone dermatitis* (Lane 1911 Medlar 1911). The term chromoblastomycosis was proposed in 1920 by Terra, Torres da Fonseca and de Azevedo. In spite of numerous criticisms this term is the most frequently used at present. The most important of these criticisms was that the word suggested a mycosis caused by budding fungus. This in 1931 led Moore and de Almeida to propose the word chromomycosis which was used in the first edition of this treatise but which does not seem to have become popular. Other names such as *errucone chromomycotic dermatitis* (Redaelli and Ciferri 1941) and *disease of Lane, Pedrosa and Gomez* (Pereira Filho 1949) have met with little approval. It would seem that the denomination chromoblastomycosis without having priority is so old and well established that its modification or replacement would lead to unnecessary confusion. This term only is used here.

Historical

The first case of chromoblastomycosis was diagnosed in Boston in 1915 by Lane and Medlar. These workers published their observation separately, the former dealing with the clinical aspects and the latter with the characters of the fungus which he had isolated and described. Five years later Pedrosa and Gomez (1920) again finding the disease in Brazil attributed it to an organism identical with the one isolated by the North American workers and Pedrosa reported having met with one case since 1910. In 1922 Terra *et al* described a new Brazilian case which resulted in the important discovery that their organism was not only dissimilar to that isolated by Medlar in the United States but also that the parasite isolated by Pedrosa and Gomez had been erroneously referred to *Phialophora verrucosa* Medlar 1911. In fact it turned out to be a form of *Acrethoeca*. Brumpt (1922) identified this Brazilian species as *Hormodendrum pedrosi*. In 1924 Carini described two new ones of *errucone*

dermatitis in São Paulo and made the point that in 1910 he had isolated from the big frog of Brazil (*Leptodactylus pentadactylus*) a fungus similar to the agent of the dermatosis.

Montpellier and Catanei discovered in 1927 in Algiers the first case of African chromoblastomycosis and also incidentally the first case of this disease recorded outside the American continent. They referred it to a new species *Hormodendron algeriensis*.

Whilst fresh cases increasingly appeared in South America and elsewhere it was not until 1933 that Wilson, Hulc and Westman reported the second North American case. This second case from Texas was like the first caused by *Phialophora terricola*. It was not until 1938 that Martin, Baker and Conant reported having found a North American case caused by *Hormodendrum pedrosoi*. In 1941 Vanbreuseghem, Vandepitte, Thys and Winder reported the first case of chromoblastomycosis in Central Africa and attributed it to *Phialophora pedrosoi*.

This short historical account emphasises the difficulties which beset workers in this field. Other fundamental contributions, whether of mycological or clinical interest, will be dealt with more appositely in the text which follows.

Importance and Geographical Distribution

In spite of the copious literature devoted to chromoblastomycosis the number of cases of the disease as yet recorded for the whole world has not appear to exceed 150-200 and it may be justly described as rare. The chief countries where it is found are Brazil, Porto Rico and Cuba. According to Pardo Castello, Rio Leon and Freepalacios who has made an extremely important contribution to the subject (1941) chromoblastomycosis is relatively frequent in Cuba. Besides the three main regions of occurrence cases have been reported from the following countries: South Africa, Algeria, Argentina, Australia, the Belgian Congo, Costa Rica, the United States, Guatemala, Japan, Java, Port Riko, Rhodesia, Russia, Dominica, Sumatra, Uruguay, Venezuela. The chromoblastomycosis though cosmopolitan is predominantly distributed in tropical and sub-tropical regions.

Etiology

Chromoblastomycosis is especially found in male adults who are being rarely attacked by it. No race is immune and it is chiefly among the hard workers that cases occur. Wounding, or wood splinters are usually involved before the disease is contracted and this is obviously related to the biology of *Phialophora*.

The Agents of Chromoblastomycosis

The pathogenic fungi isolated from cases of chromoblastomycosis comprise three species within the unique genus *Phialophora* (Mellor 1911) and Emmon 1944. They are *Phialophora terricola* Mellor 1911

Phialophora pedroni (Brumpt 1911) n. comb. Emmons 1944 and *Phialophora compacta* (Carrion 1913) n. comb. Emmons 1944. The genus *Phialophora* attributed by some to Medlar and by others to Thaxter was first described by Medlar in 1915. In this paper it was clearly stated that Medlar had consulted Thaxter but it is equally clear that Medlar claims the credit for the erection of the genus *Phialophora* and the species *errucosa*. One of the figures from the work in question has the legend *Phialophora errucosa* Medlar a fact which seems to be conclusive.

On the other hand Emmons who willingly concedes the genus *Phialophora* to Thaxter (in *Henrici, Mykologia and Actinomyces* 1948) attributes the revision of the genus to three authors namely Burford H. W. and Emmons (1944) though it is evident from the very title of the work that it is to Emmons that we owe this revision. The courtesy shown by the great American mycologist to his co-authors is understandable but not generally acceptable and the correct designation would appear to be *Phialophora* (Medlar 1915) emend. Emmons 1944 and not *Phialophora* (Thaxter 1915) emend. Burford et al. 1944.

The *Phialophora* species are *Dematium* with two forms of imperfect reproduction which may be found simultaneously or separately.

1. Unicellular phialospore scarcely or not pigmented may be produced in phialides—type *Phialophora*.

There may be erogenous pleurogonous or acropleurogonous reproduction upon erect conidiophores of dark spores (type *Aerobolus*) or upon erect terminal or lateral conidiophores of spores in small chains (type *Hormodendrum*).

This larger conception of the genus *Phialophora* is the result of the better knowledge of the mycology of *Actinomyces*. In view of the apparent conflict between Medlar's finding (1915) that the disease was caused by a species with a single form of reproduction and those of Terra et al. (1921) who demonstrated the existence of another species with another form of reproduction it became evident eventually that several forms of reproduction could co-exist in the same species. If the three known species are accepted as *errucosa*, *pedroni* and *compacta* then apart from *errucosa* which is unanimously attributed to the genus *Phialophora* the other species are variously distributed amongst the genera *Hormodendrum* (or *Chelosporium*) *Phialophora*, *Fonsecaea* etc. Thus in the literature *Hormodendrum pedroni* is readily found as *Phialophora* or *Fonsecaea pedroni*.

It is important to recall that in 1937 Conant established on morphological basis that the genus *Chelosporium* Lagerberg and Melin 1937 comprising various species discovered in wood pulp must go with *Phialophora* Thaxter 1915 with which it shares characteristic reproduction by

In view of *Hormodendrum* correct and not *H. adei* C. B. d.
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phialides of *Phialophora americana* Nanf is thus synonymous with *Phialophora terricola* and the following *Phialophora* species pass into the genus *Phialophora*—

<i>Ph. f. digit</i> (Lagerl. and M. lin.)	<i>Ph. M. l.</i> (Nanf) Conant
Conant	<i>Ph. repens</i> (Davidson) Conant
<i>Ph. Brunneoven</i> (Davidson) Conant	<i>Ph. Richardson</i> (Nanf) Conant
<i>Ph. Lagerberg</i> (Melin and Nanf)	
Conant	

It is of interest that 1938 Martin's study of complement fixation established the antigen similarity of a strain of *Phialophora americana* with several strains of *Ph. terricola* isolated from human sources whereas other *Phialophora* strains isolated from wood pulp but differing in morphology from *Ph. terricola* belonged to a different antigen group.

The Cultures—Macroscopic

The morphology of the three species of fungi using hummocky mycelium varies according to the culture medium and the origin of the strains. In any case they differ but slightly from one another but it is generally agreed that colonies of *Ph. compacta* grow more slowly than those of the other species.

In general the colonies are in colour from very dark brown to olive black. They take the form of a flattened dome with a frequently mammillated summit lessening in slope towards the periphery and terminating with a regular or fringed edge. Concentric or radiating furrows may be visible.

The colonies are colored with a more or less prominent light or dark brown down. For identification of the species macroscopic examination is required as the aspect of the cultures gives an insufficient indication. Examination is best carried out with hanging drops which leave the spores in their proper place whereas teasing out completely disturbs them. Cultures on glass slides are unsuitable for study for the hard and very tumid colonies cannot readily be compressed between slide and cover slip. Cultures on hair isolated *in vitro* (Vanbreughem 1950) are well suited for microscopic study of the morphology of the *Phialophora* species.

The Cultures—Microscopic

The vegetative mycelium of *Phialophora* does not vary from species to species. It is composed of rectilinear or undulated hyphae with thick and dark walls. The hyphae are segmented and branched and their diameter is 1.1–3 μ . The chlamydospores recall the rounded cells found in truffles; they are rare, spherical, thick-walled and 8–12 μ in diameter. Some chlamydospores are partitioned into 2, 3 or 4 cells.

The forms of reproduction of three pathogenic species are given

1 *Phialophora terrucona* Medlar 1913 (syn *Ladophora americana* Vannfeld 1927 *Phialophora macrospora* Moore and Almeida 1934)

The reproductive structures are contained in lateral or terminal phialides. The conidia or phialospores are borne at the base of the funnel formed by the collar of the phialide. The phialospores are not in small chains as in the next species but are aggregated in rounded masses held together by a viscous substance. The phialides (3-4 μ wide by 4-11 μ long) occur singly or in groups. The phialospores are oval and 1.5-3 μ . Besides this mode of reproduction there may rarely appear the same mode of reproduction as in *Ph. pedronii*.

Phialophora pedronii (Brumpt 1922) Frimmon 1944

Synonyms—

<i>Hormodendrum pedronii</i> Brumpt 1922	<i>Compharia pedronii</i> De L. 1935
<i>Acretheca pedronii</i> La Fontaine and Leno 1933	<i>Hatyloide monophora</i> Moore and Almeida 1936
<i>Hormodendrum algeriense</i> Montpelier and Citane 1927	<i>Phialoconidiophora (ajj) pedronii</i> Moore and Almeida 1934
<i>Trichosporium pedronii</i> Ota 1928	<i>Hormodendroide pedronii</i> Moore and Almeida 1936
<i>Trichosporium pedronii</i> Laryon 1929	<i>Trichosporium pedronii</i> Carmon 1944

This species generally reproduces by conidia borne on terminal or lateral conidiophores on the aerial mycelium (type *Hormodendrum*) or by terminal or lateral budding (?) of the vegetative hyphae (type *Acretheca*). In the *Hormodendrum* type the conidia develop basipetally at the extremity of a conidiophore and remain in chains but each conidium may act as the starting point of a small chain of secondary conidia which in turn may yield tertiary conidia. The conidia from which conidia have originated are modified taking the form of an echinon (shank haped spore of American writers). The uncellular conidia are 3-4 μ long by 1.5-3 μ wide are of dark blue colour and are connected to one another by thick interstitial structures. In the *Acretheca* type the conidiophore resemble a knotted stick yielding a chain of knot-like rounded and dark blue uncellular conidium.

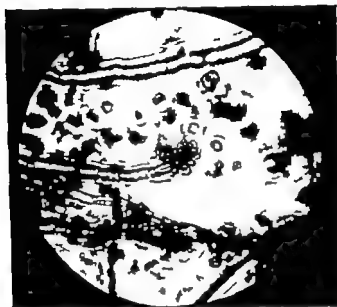
Apart from the above type of reproduction there may rarely be found in *Ph. pedronii* typical phialospores as in *Ph. terrucona*.

3 *Phialophora compactum* (Carmon 1933) Frimmon 1944

Synonyms—

<i>Hormodendrum compactum</i> (Carmon 1933)	<i>Phialoconidiophora compactum</i> Moore and Almeida 1934
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In this rare β is the xth phorbol unit ($1 \leq x \leq 12$) form compact masses of long, branching, hairs arranged on lateral or terminal exodiphores.



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A. ordinatus Curran (1914) this species has been found in one locality in Port Jervis.

Symptomatology

Chromoblastomys usually starts with a pustule or nodule confined to lower extremity. Frequently the patient call attention to an anterior transverse. The foot and leg are especially affected but in order of frequency come the hands, the forearm and the arm, the neck, the shoulders and the buttock. The lesions with rare exception are unilateral.

The pustule or nodule forming, the primary lesion may become pruriginous. This lesion enlarges, congests, runs, then becomes dry and crusty. Its peripheral extension is somewhat limited and is mainly by multiple autoinoculations around the primary lesion that the latter enlarges and resembles after several years a cauliflower formation. The epidermis generally thinner at the surface of the lesion bleeds and ulcerate easily. In other cases the epidermis is covered with scales and

scabs. Young lesions are generally limited, violaceous or dark red in colour and very infiltrated. After frequent ulceration infection progresses regularly and the lymphatics become blocked. At this stage the patient emits a repulsive odour.

Metastasis is exceptional but not unknown. The lymph nodes are usually left alone except in cases of secondary infection. Merime (1935) claims to have observed sequestrated cells in lymphatic channels.



FIG. 4

(1) Corneal lesion (2) Chromoblastomycosis (3) Verrucous dermatoma (4) Verrucous carcinoma

which also contained tubercular bacilli. A strain of *Horowitzia* in roseum was recovered from culture.

Evidently the symptomatology of chromoblastomycosis is not yet well established, which is to be expected in view of the relatively small number of known cases. Generally speaking the lesion is circumscribed, a verrucous dermatoma, and Carrion (1941) made the interesting observation. The more highly pigmented the lesion the less it will be noticed by the surrounding skin.

There is however a tendency toward a fuller study of the symptomatology which may well bring about a modification of the definition of chromoblastomycosis, increasing the differential diagnosis and ultimately result in the discovery of a greater number of cases.

Carrion and Silva (1947) distinguished five types—

(1) A nodular type at the beginning the nodules are putrid, deeply coloured with a smooth verrucous or squamous surface.

(2) A tumoral type with papillomatous masses sometimes like cauliflower.

1 Verrucous type in which the nodul or tumoral masses are hyperkeratoid

4 A blotchy type rare squamous stains slightly elevated deeply coloured

A central type characterized by a healing of the centre of the lesions while they spread at the circumference. The centre is replaced by a trophic scar

A study of 31 cases in Cuba by Pardo Castillo, Irujo Leon and Trespiñon (1941) has resulted in the following classification—

1 Verrucous type	1 case
Tuberculous type	4 cases
3 Syphilitic type	4 cases
4 Psoriasisiform type	4 cases
Central and elephantiasis type	4 cases

The *Phialophora* species can cause other clinical lesions besides the localized chromoblastomycosis from a generalized onychomycosis which the cultures were opaque friable and brown. Irujo Leon (1939) isolated a fungus which he identified *Acretheca pedronis*. Again from onychomycosis by transference, Torula Jeannelae Langeron 1931 the genus *Phialophora* showed at the same time that *Phialophora* species may be agent of mycetomycosis

Histopathology

The histopathological picture is that of an infectious granuloma. The epidermis is generally hypertrophied with hyperkeratosis not black scanthous and elongation of the interpapillary processes. Polynuclear leucocytes sometimes infiltrate into the epidermal layers and can even form small abscesses there. In the dermis occurs abundant infiltration of lymphocytes plasmaocytes large mononuclear polynuclear eosinophilic Hassel bodies epithelioid cells giant cells of Langhans and foreign body type respectively. This infiltration whether localized or diffuse is mainly developed in the superior dermal parts the papillae of which are hypertrophied. Frequently milium bodies are encountered rarely necrosis. The organisms isolated or in groups are free in the dermis or epidermis in the dermis they are often included in giant cells

Treatment

1 Surgical

All workers admit that either extraction or electrocoagulation of the initial lesions (followed by hyperextensive gentian violet application according to Pardo Castillo 1941) must be employed. However even in little and old cases relapses are frequent

2 Chemotherapy

There is no agreement that the role of potassium iodide administered per os for long time and in very strong doses (up to

10-15 μ per day) and of sodium iodide intravenously (1 μ at the start reaching 9 g after some month). Martin Baker and Conant (1936) have in one case used iontophoresis with copper sulphate with some success.

3 Radiotherapy

This has long been used. Pardo Castello *et al* (194) obtained a vit in superficial form by administering 600-1 500 r filtered by 1 mm of aluminium.

Besides these therapeutic measures designed to suppress the disease, secondary therapeutics for the disinfection of tissues attacked will take



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a predominant place in all analyses of the data. Even in the stage structure of iodine added to 10-20 per cent chloride and applied to all brines, about some reduction of the k ions.

The problem is really one of early diagnosis. Since until now only extraction and electrocoagulation have given definitely result in the young form, an early diagnosis is obviously very important.

Promotions

(chromoblastomycosis) is a disease of very long duration. So far as is known up to recent years most lesions have been male lesions.

is standing, and it is known when the lesion was present for 40 years. The disease is therefore *quodammodo* benign one. It becomes of importance only when the tissues are affected the lymphatics blocked and a certain degree of elephantiasis is developed. Sometimes in these cases amputation of limb has to be considered though in general it may well be that local cure, chemotherapeutic measures directed against secondary infection and partial extraction by limited and an-cure electrocoagulations may delay an operation or postpone it indefinitely.

Differential Diagnosis

Though the symptomatology of net blighted chromoblastomycosis may be sufficiently typical to require only simple laboratory confirmation, this is far from true at the onset of the disease. There may be confusion with leprosy, tuberculosis, jaw syphilis, kashanirrus monilia and Gilchrist's disease. At certain stages a run the obscure monkey foot and *erruconis lymphaticus* (or *lymphaticus verrucosae cutis*) might suggest a diagnosis of chromoblastomycosis.

Mycological Diagnosis

Four methods of approach may contribute to a diagnosis of chromoblastomycosis—

1. *Clincal*. As already mentioned diagnosis is difficult except when the disease is fully established and it is in vain to rely wholly upon laboratory examination.

Examination of the Pathogen Fungus. Scales Epidermal Debris and Tissue. This may be carried out either by biopsy and an aseptically pathological examination or by direct examination of crust or serum from the surface mounted in potash.

The organisms concerned take the form of rounded bodies in the tissues occurring singly or in large numbers and surrounded by a thick dark brown membrane. They multiply by cell division and not by budding. The first division yields two cells which also tend to be rounded and these may divide again without separation soon these filia which are not obviously invaginated contain a granular olive brown protoplasm and are about 10μ in diameter. In sections stained with Gram's the cell wall appears brownish green.

Those who first described the disease gave the name scleroma or sclerotic cells to the fungal cells found in the tissues. This designation is controversial. Langeron prefers to call them fungoid cells. Emmon appears to have adopted the term hamydozoa which applied to tissue forms is certainly an innovation. It is interesting to note that de Ara Lazo Mello and Cury (1947) found in rats subjected to intratesticular injection nine months previously granules made up of dark rounded cells surrounded by cellular granules of actinomycosis. Again Wiedman

and Iosenthal (1931) found tub like granule in a human skin from which they isolated *Hormodendrum pedrosoi*.

In scales the fumigoid forms may be found to have given rise to filament.

In section strongly coloured fumigoid form are easily found either intra or extra cellularly in the dermis or in the superficial hyperkeratotic strata.

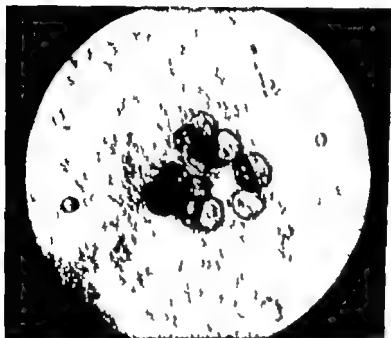


Fig. 1. Histological section of a human skin showing the presence of *Hormodendrum pedrosoi* in the dermis.

In some pathological examination is concerned not only of the evidence to be gained of a specific histological picture which is that of all infectious granuloma but because it permits the recovery of fumigoid forms *in situ*. In our specimen the form indicates the presence of chromoblastomycosis and the organisms involved can be cultivated in culture.

Culture isolation in culture carried out in ordinary temperature in Sabouraud test medium using either cork or small fragment of tissue. Binford, Hess and Fennons (1944) failed in one case when the fragment of tissue used had probably been subjected to 9 per cent ethyl alcohol and they recall a similar failure by Wiedmann and Rothel.

Experimental Inoculation

1) It is first necessary to emphasize that animal inoculation does not result in a reproduction of the disease as it is encountered in man. Human inoculation has never been attempted by Takahashi (1937) who successfully inoculated an individual already subject to chromosomal changes by making the inoculation in a healthy region and also an individual compensated for the disease.

As seen in *Uca*, as shown the intradorsal or subventrocompunction of cultures end with the formation of nodules or bryozoan which is found the same funal form as in the human disease.

3. Intracutaneous nodules mostly be studied in the rat. In inflammatory arthritis first appears followed by abscess within some months.

4 Intraperitoneal injection in the rat or mouse provokes the formation of nodules on the mesenteria and other intraperitoneal or an

8 By the intraperitoneal route de tre L. V. Lin and Cury (1947) a culture obtained from the stubbed granule de eloped in its testicle obtained a generalized lewis in the mouse. These would appear to be exceptional.

Discussion

Chromoblastomycosis is a mycosis caused by accidental injection with several species of *Phaeophypha*. Diagnosis is readily established by observation in the tissues of fungus and fungus also called leukotrich cells or chlamydospores. Therapeutic measures have been but little effect upon the disease which is of long duration.

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Coccidioidomycosis

Definition

Coccidioidomycosis is an infectious disease caused by *Coccidioides immitis*. Very benign and scarcely noticeable at the time when it is first contracted (reparatory or primary form) it may exceptionally give way to a chronic form (secondary or progressive) characterized by a varied symptomatology and a high death rate.

There is in fact very little controversy about the name given to the mycosis the synonyms which are generally applied represent only certain phases of the disease, or indeed particular aspects of certain phases. Some of these are Coccidioidal granuloma (Valley Fever, Desert Pneumonia, San Joaquin Fever) and less frequently Posada's Wernicke's Disease. This last name is applied to the disease as a whole and not merely to one aspect of it.

Historical

In 1891 Posada and Wernicke discovered in Argentina the first case of disease which they considered to summat a mycosis and which they attributed to a protozoan. They published their results separately in 1892. The second and third cases were found in 1896 in California by Purford and Gilchrist who named the causal agent *Coccidioides immitis* and also considered it to be protozoan. Ophuls and Moffitt (1900) established by culture in the third American case the fungal nature of the organism. In 1918 Giltner discovered the first case of coccidioidal granuloma in a bovine at San Diego, California. In 1931 Stewart and Meyer first isolated *Coccidioides immitis* from the ground at a place where four cases of progressive coccidioidomycosis had been detected. Dickson in 1937 proposed the new term coccidioidomycosis to designate at the same time the primary form (Valley Fever) and the secondary form (coccidioidal granuloma) of the disease. In 1941 Emmons isolated *C. immitis* from desert rodent and in the same year with Ishburn described *H. pl. sporangium parvum*. These discoveries clarified some points of the etiology of coccidioidomycosis but at the same time added their quota of confusion.

Importance and Geographical Distribution

Although the first case of coccidioidal granuloma was described in Argentina it is now quite certain that coccidioidomycosis is almost

completely limited to the desert regions of the south western United States namely California Arizona Texas and New Mexico. Only four cases have been reported from South America all of them from the Chaco region of Argentina. One case has been reported by Fernel from the Hawaiian Isles three from Italy and possibly in 191 Hartmann and Schoon saw one case in Holland.

In the United States quite a few cases have been described outside the principal endemic zones but more often than not it has been possible to relate the occurrence to a previous visit by the patient to one of these zones.

It would be difficult to estimate the number of cases of primary coccidioidomycosis on record as they are so very numerous. The secondary form is by contrast always rare. According to Forbes and Be telecreutz (1946) 8 000 cases of primary coccidioidomycosis were diagnosed in soldiers during the last war.

Coccidioidomycosis is a disease of all ages without predominance of sex or race. However the serious form is usually found in men and mainly in coloured races e.g. Negroes Indians Mexicans and Philipinos. In the latter it may be particularly serious. According to Smith Beard Whiting and Rosenberger (1946) the progressive form occurs four times more frequently in men than in women further Mexicans are 31 times more likely to have it than whites negroes 14 times and Philipinos 180 times.

Children living in endemic areas apparently contract at an early age a benign form of the disease detectable by the cutaneous test which immunises them against further attacks. Young adults who are not immunised and who enter the endemic region appear to be most susceptible.

Laboratory infections caused by the inhalation of cultures may arise anywhere. Smith and Harrell Jr (1948) reported 17 cases of laboratory infection five of them with sub clinical manifestations and one which proved fatal. Varro (1948) reported a case which occurred in England.

Etiology

Two points appear to be important in an exact conception of the etiology of coccidioidomycosis—

- 1 This highly infectious disease is not contagious and patient having one or other form of the disease do not transmit it. This essential fact is in no way invalidated by the fact that laboratory animals may possibly be infected from patient sputum. Rosenthal and Poutien (1946 1947) have in fact successfully inoculated the parasitic form of *Coccidioides immitis* by suffusing the trachea of Guinea pigs with pus sputum and scraping from coccidioidomycotic granuloma. It is noteworthy that the inoculating material kept at 1°C for 110 days retained its infective power. In spite of the conclusion of the workers who believe that coccidioidomycosis must be regarded as a contagious disease until the

contrary has been proved the absence of any known case of interhuman contagion points conclusively to the non contagious nature of coccidioidomycosis.

It is known that dust from the soil is an important factor of infection if not the only one. This fact emerges from geographical consideration, from periodic epidemiology and from experimentation.

A Geographical Considerations

Coccidioidomycosis is a disease of the desert regions of the southwestern United States. Smith Beard Whiting and Rosenberger (1946) have shown that in the northern part the annual rate of infection is only 0.8 per cent whereas it is 20 to 25 per cent in the south. This clearly established the possibility of a causal relationship between the sandy nature of the soil and the development of the disease.

B Epidemiological Considerations

These have arisen comparatively recently and are based essentially on the mass experience which resulted from the installation during the 1940-41 war of airfield in the south west of the United States. In 1940 Smith had already demonstrated that the disease is purely periodic that it reaches its maximum during the dry seasons of summer and autumn when work goes on in the field and had concluded that transmission of the disease takes place as dust. Eventually Smith Beard Rosenberger and Whiting (1946) by means of cutaneous tests with coccidioidin demonstrated that the number of new cases could be reduced by diminishing the formation of dust by the development of meadows by the tarring of roads or by the dispersion of oil. Smith *et al* believe that dust blown by the wind distributes the infectious spores and that infection occurs through the respiratory organs. They consider that rain in winter is favourable to the vegetation on which *Coccidioides immitis* is developed, fact yet not proved and that in summer it combats infection by diminishing the dust.

C Experimentation

In 1932 Stewart and Meyer for the first time isolated *C. immitis* from the soil in Delano, a place near which four cases of progressive coccidioidomycosis had been reported. In 1941 Davis Smith and Smith isolated the parasite from the soil at San Benito California. In 1941 Enmons isolated *Coccidioides immitis* five times from 170 samples of earth collected in the region of San Carlos Arizona. According to Enmons (1947) the technique for isolating *C. immitis* from the soil is as follows. The soil is first emulsified in 30 per cent solution of sodium chloride shaken then the large particles which accumulate at the surface are removed. It is allowed to decant for an hour when the supernatant liquid is collected and diluted to the sodium chloride concentration of physiological serum. This is then centrifuged and cultured or else the sediment

is used for inoculation. In practice the guinea pig gave the best result for inoculation.

Thus a number of investigations have demonstrated the existence of *C. immitis* in the soil and explain the infectious power of dust. However there still remain the questions of how the organism happens to be in the soil whether it stays there and whether it develops there. Some very stimulating new data has resulted from important studies undertaken by Emmons and Ashburn (1941). Emmons carried out his observations at the San Carlos Indian Reservation, Arizona, a region in which coccidioidomycosis was unknown but where Irons and Saylor and Parr (1942) had obtained a positive cutaneous reaction to coccidioidin from more than 90 per cent of the Indian school children. This observation had appeared to be so curious to Irons and his co-workers that Irons and Callacher had followed up with a test upon New York children at the same time (1941) using the same antigen and had obtained a completely negative result.

In the same region of San Carlos Emmons caught 203 wild rodents and was able to record that 15 per cent of 14 mice (pocket mice i.e. *Perognathus baileyi*, *P. penicillatus*, *P. intermedius*) and 1 per cent of 29 rats (hangaroo rat i.e. *Dipodomys merriami*) were infected by *Coccidioides immitis*. This organism produces in these rodents a pulmonary mycosis characterized by nodules distributed in the peripheral rather than in the central parenchyma and these nodules may be calcified. A curious fact is that 11 captured specimens of *Peromyscus eremicus* showed no sign of spontaneous infection by *C. immitis* though this animal is very sensitive to the experimental inoculation of the disease.

Besides *C. immitis* Emmons isolated another fungus belonging to the genus *Haploporangium* Thaxter which he called *Haploporangium parvum*. This new organism produces microscopic granular masses in rodents of the genera *Perognathus* (69 per cent of 14), *Dipodomys*, *Citellus* and others.

From *Haploporangium parvum* Emmons was able to prepare haplosporidin which gave 29 positive reactions out of 33 individuals reacting positively to coccidioidin. This interesting pathogen will again be referred to when dealing with haplomycosis.

Certain hypotheses emerge from Emmons's observations and an obvious first question is whether though *Haploporangium parvum* has not yet been isolated from man this organism is not responsible for a certain number of positive coccidioidin tests. In fact it is easy to suppose that *H. parvum* which causes minute lesions in rodent may be able to produce inconspicuous disease in man. What is more in considering the existence of *C. immitis* and *H. parvum* in rodent it is tempting to speculate upon the existence of an animal reservoir. However it is still obscure whether like man these animals are merely receptacles or whether they are indeed essential for the maintenance of the organism in the soil.

It is noteworthy that *Perognathus* and *Dipodomys* are rodents which

are restricted in their distribution to the desert region of the southwestern United States where *Peromyscus* which is not in nature infected by *C. munitis* spread in northern regions of America. A *Peromyscus* under laboratory conditions is rapidly killed when infected whilst *Perognathus* and *Dipodomys* are merely chronically affected it may be wondered whether the role of *Peromyscus* is not limited by its susceptibility to *Coccidioides munitis*.

Thus several important points remain to be cleared up especially (i) the exact role of the soil in maintaining the infection and (ii) the possible intervention in man of *Hayfever* as a possible agent of fibre reactions to coccidioidosis. The possibility immediately suggests itself of using this pathogen as an immunizing agent against coccidioidomycosis.

Pathogenic Agent

The mycological study of coccidioidomycosis is simplified by the fact that only one pathogenic agent for it is known namely *Coccidioides munitis* Riford and Chricht 1896.

The genus *Coccidioides* at present represented by only one species has the following synonyms—

<i>Posseltia</i> Canton 1894	<i>Crichtum</i> (pro parte) near Baical 1911 non Link
<i>Ondium</i> or <i>Ophid</i> 190 non Link	<i>Scopulariopsis</i> (pro parte) near Ota 1934 non Ramet
<i>Blasporia</i> in Hartmann 1912(?)	<i>Climacopora</i> near Castellani and Jacopo 1913 non Berkeley and Curtis
<i>Mycoderma</i> (pro parte) near Brumpt 1913 non Persoon	
<i>Blasomycodes</i> Castellani 1926	
<i>Paracoccidioides</i> Fonseca 1924	

The species synonyms are numerous but unimportant since the binomial *Coccidioides munitis* is the only one used in recent publication they are—

<i>Coccidioides pyogenes</i> Riford and Chricht 1896	<i>Scopulariopsis merionum</i> Ota 1934
<i>Posseltia ceteriformis</i> Canton 1894	<i>Crichtum dermatitidis</i> Baical 1911
<i>Ondium protocoides</i> Ophid 190	<i>Coccidioides ceteriformis</i> Moore 1913
<i>Ondium pyogenes</i> Ophid 190	<i>Crichtum munitis</i> Agostini 1913
<i>Ondium munitis</i> Verdun 1907	<i>Crichtum Louisaenoides</i> Castellani 1913
<i>Blasporia</i> in Achon Hritman 1912(?)	<i>Climacopora metaxopora</i> Castellani 1913
<i>Synonyma munitis</i> Mello and Fernandez 1918	<i>Coccidioides munitis</i> or <i>typicus</i> Ciferri and Redaelli 1936
<i>Mycoderma munitis</i> Brumpt 1913	<i>Coccidioides munitis</i> var <i>papa</i> Ciferri and Redaelli 1936
<i>Blasomycodes munitis</i> Castellani 1926	<i>Coccidioides munitis</i> or <i>metaxoporus</i> Ciferri and Redaelli 1936
<i>Paracoccidioides munitis</i> Marzani and Fonseca 1924	

This parasitic fungus has the curious distinction of having been first described in Argentina where it is exceptional and of having been mistaken for a coccid by the first observers (Porcia 1897 Wernicke 1897) Rixford and Gilchrist (1896) who named it considered it to be a member of the protista. For this reason the validity of their denomination has been disputed though objections cannot really be supported. Further some would attribute the first description to Stiles (*Coccidioides immitis* Stiles 1896) who had been consulted by Rixford and Gilchrist this is incorrect for Stiles had merely given advice of a general nature. It was not until 1900 that Ophub and Moffith demonstrated the fungal nature of the pathogen by cultural method.

Parasitic Form of *Coccidioides immitis*

The organism appears in tissues or secretions as circular bodies named spherules or sporangia in which occur a very variable number of small spores called endospores or sporangiospores. Rupture of the sporangial membrane liberates the endospores into the surrounding tissue where they are found free or phagocyted. The endospores are 1 to 4 μ in diameter and are usually thought to have only one nucleus. They develop into sporangia which at maturity are 20 to 60 μ in diameter and sometimes reach 80 μ . Various factors determine the diameter attained by the sporangia such as the strain involved nature of the tissue parasitized and the host species. In mice and guinea pigs they are particularly large. The sporangia approaching maturity generally exhibit a large central vacuole so that in mice for example where this is particularly clear the protoplasm may be reduced to a thin multinucleate layer closely adpressed against the sporangial membrane. This protoplasm is divided by radial and concentric cleavage planes into multinucleate fragments which eventually lead to the formation of uninucleate endospores. If it should happen that fewer protoplasmic cleavages occur the sporangium may be found to contain rounded multinucleate masses instead of a large number of uninucleate endospores.

The wall of the spherule may be 2 μ thick and usually has a smooth outer surface. It has however been observed to be covered with excrescences and this has led Hennes to speak of actinomorphic form.

All workers are agreed that the spherules of *C. immitis* do not bud. However Delamater and Weed (1918) have claimed they have been budding not only in their own isolated strain but also in the cells stained by other workers. This observation must be accepted with extreme caution.

Emmons (1914) showed that the endospore nucleus has the same morphology as characteristic fungal nuclei. It is surrounded by a delicate nuclear membrane and possesses one eccentric nucleolus.

Culture Morphology : Macroscopic

Coccidioides immitis grows rapidly on Sabouraud medium. After two days at 37°C slightly elevated circular colonies which are quickly

covered with a whit down which browns on ageing may appear. Often well developed in the centre this down is usually less prominent at the periphery. The under surface of the colonies is dark near the centre. There are somewhat large variation in the aspect of the colonies according to the strain some being able to form a lemon yellow pigment and others remaining membranous and smooth.

Culture Morphology - Microscopic

The vegetative mycelium is composed of segmented hyphae of diameter $1-4\mu$. Fennell (191) drew attention to specialized sporophores borne upon hyphae characteristic of young cultures which in his view were typical for *C. immitis*. Lateral branches appear upon the vegetative hyphae of the same width as the upon which they arise they rapidly double their width however and may produce secondary branches. Each of these becomes subdivided into short segments 2 to 4μ long in which the protoplasm condenses these are the arthrospores or chlamydospores of Fennell. In older cultures chlamydospores appear to occur in various regions but it is probable that the typical sporophores are similarly formed.

Protoplasmic condensation within the arthrospores may occur at the centre or against one of the septa if the protoplasm of two neighbouring arthrospores condenses against common septum the usual symmetry is disturbed. The arthrospores which readily break up in old cultures remain together grouped in pairs or small groups in young cultures.

Baker and Urah (1941) obtained from old cultures spherules analogous with the tissue forms containing endospores and of diameter not exceeding $10-20\mu$. Their occurrence must however be regarded as exceptional.

Symptomatology

It was not until 1937 that Dickson established that San Joaquin fever and coccidioidal granuloma are of one and the same origin the former being merely a stage of the latter. The same worker proposed the distinction between the primary form of coccidioidomycosis corresponding to San Joaquin fever and the secondary or progressive form which is coccidioidal granuloma.

1 Primary Coccidioidomycosis

This form appears 10 to 14 days after exposure to dust in endemic regions. It is characterized by pulmonary symptoms which are scarcely noticeable cough usually without expectoration pleural pain slight temperature (39°C) sthenia anorexia headache night sweating. In the majority of cases normal health is restored in one or two weeks. Sometimes generalized and temporary maculous rash appears. Radiological examination made at this stage reveals in 80 per cent of the cases pulmonary changes which may include—

1. Hilar infiltration

2. Infiltration of the pulmonary tissue in the middle or lower regions of the lungs

3. Single or multiple nodular lesions chiefly situated in the middle or lower regions. These nodules may disappear or become cyst like cavities with thin partitions which either soon vanish or may persist for several years and become calcified. In nine cases out of ten the cavities are apical and in one eighth of the cases they are apical

4. More rarely mediastinal and hilar adenopathy occurs

5. In one fifth of the cases a pleural effusion may be noted

The form of the cavity associated with pulmonary lesions has given rise to a somewhat abundant literature. According to Smith Beard and Tailor (1918) these cavities which may be present from the beginning usually disappear after three or four months but are able to persist for as long as ten years. They cannot be regarded as belonging to the progressive form of the disease. Mostly they are well tolerated (as for example in the

case of a soldier rested on account of a lesion of this nature who indulged violently in sport without any prejudice to his recovery) sometimes however they are complicated by hemoptyses or hydropneumothorax (about 10 per cent of the cases). The same authors have observed cavitation in about 8 per cent of hospital cases which fact does not establish the real frequency of pulmonary cavitation for the greater number of patients are ambulatory. On the other hand a mixed occasional and tubercular infection was found in 7 out of 24 cases.

From 10 to 20 days after the beginning of respiratory symptoms

allergic manifestations appear in about 5 per cent of the patient. They are either *erythema nodosum* localized particularly in the legs but also in the arms buttocks thighs and scalp or *erythema multiforme* localized in the hands face and neck. Possibly there is coexistence of the two forms. The name Valley Fever was applied to these forms though the whole of the primary forms tend to be lumped together under this description. The old appellation Desert Rheumatism arose from the supposition that though it be of an inflammation of the articulations of the knee and instep. Occasionally a phlyctenular conjunctivitis is found.

In 100 cases of primary occidiosis of Willett and Willett (1918) have observed the following clinical manifestations: fever less than a week 50 per cent thoracic pain 3 per cent dry cough 6 per cent sputum blood streaked 3 per cent articular symptoms 8 per cent *erythema nodosum* 4 per cent *erythema multiforme* 1 per cent malaise 43 per cent anorexia 40 per cent loss of weight 2 per cent splenomegaly 1 per cent

In 60 per cent of cases exposure to infection due to a fly is not followed by symptoms but unnoticed illness develops and recedes itself by positive reaction to cochenillidin. However not all the experimental infections develop clinically detectable or unapparent illness. Smith Beard, Roenbergs and Whiting (1916) observed that only a very small percentage of individuals (1 to 4 per cent) at first reacting negatively to the already

become primary reactors. Thus we do not know the exact sensitivity of man to *Coccidioides immitis*. But what is certain is that only 1 per 1 000 of those who have had the mild primary form develop the secondary progressive form which is nearly always fatal.

Primary non-pulmonary forms of coccidioidomycosis have been described (Conant *et al.* *Manual of Clinical Mycology* 1947). These cases seem to be more difficult to distinguish from coccidioidomycosis can develop without symptoms and that moreover it is not yet proved that only those previously having an apparent primary form develop the progressive form.

3 Secondary or Progressive Coccidioidomycosis

This form corresponding to the old coccidioidal granuloma appears in 1 per 1 000 of those who have exhibited the primary phase. It is usually fatal. The progressive form appears during the weeks or perhaps months which follow the primary phase. It lasts from few months to a year or more. Symptoms are observed a light fever, anorexia, asthenia and rapid diminution in weight. The lungs exhibit a very pronounced pulmonary condensation and infiltrations reminiscent of tuberculosis. Bony lesions with the appearance of cysts are found particularly in the ribs and the small bones of the hand and feet. The lymph nodes, joint, skin, subcutaneous tissue, meninges and the brain may be involved. Meningitis complicates the progressive form in 20 per cent of cases.

In contrast with what occurs in South American blastomycosis, lesions of the gastrointestinal tract are exceptional in coccidioidomycosis. Daemling (1949) has, however, recently reported two cases amongst coloured people infected in San Diego.

Histopathology

There is little information on this subject. As indicated by the name formerly given to the progressive phase of the disease the central lesion is granuloma. The nodules resulting from the accumulation of these granulomas may abscess or calcify. In all cases the picture is that of a tuberculous lesion at the corresponding site. The only distinction between coccidioidomycosis and tuberculosis is the presence in the former of the characteristic spherules found free or within giant cells.

Treatment

Symptomatic treatment is effected with the primary form. The presence of cavities within the lungs demand care though usually they respond well. Treatment of the progressive form has up to the present been completely disappointing. No result has been obtained by the use of iodides, sulphonamides, penicillin, gentian violet or specific metals. Jacobson (1932) recommended the use of an extract from *C. immitis* consisting of a mixture of a filtrate from culture and one from macerated

organisms. Injection of this has brought about an improvement and even cure in certain cases.

Prognosis

Extremely favourable in the primary form; it is very grave in the secondary form, which almost invariably terminates fatally within some month, a year or sometimes longer.

Differential Diagnosis

Diagnosis of coccidioidomycosis must be considered in connexion with all patients living in the endemic zones or who have resided there. It is also necessary to remember that the handling of cultures is very dangerous and that all those who work in laboratories where strains of *C. immitis* are kept are susceptible to infection. The three cases in Naples reinforce the possibility of infection outside the endemic regions. Primary coccidioidomycosis may obviously be confused with any of the benign respiratory diseases. The progressive form is reminiscent of a number of diseases too large to enumerate. It can be stated simply that it is capable of presenting the symptomatology of the numerous diseases involving neoplasm or of microbial or fungal origin.

Diagnosis

Coccidioidomycosis may be diagnosed in four different ways: by microscopic examination, culture, animal inoculation and by cutaneous tests and serology.

1. Examination of the Pathogen in Exudates and Tissues

Pus or sputum, often scarce in the primary form, may be examined between slide and cover slip or in a drop of 10 per cent caustic potash. The same may be carried out with pleural liquid or gastric content. The characteristic cell already described may be found with some difficulty.

According to Jacobson (1949) diagnosis may easily be obtained by diluting pus or sputum with physiological serum between slide and cover slip, then sealing with paraffin. Germination of the pherula occurs within a few hours and germ tube appears in all directions. Diagnosis in this manner requires one or two days.

It is of interest that Willet and Werns (1941) could demonstrate the presence of the parasite by direct examination of sputum in only 74 out of 100 cases, whereas by culture it could be isolated from 84 per cent of the cases.

Section may be stained with iron haematoxylin or Gram.

2. Isolation in Culture

The cultural requirement of *Coccidioides immitis* is simple. Arns Leão and Cury (1950) showed recently that this fungus is auxotrophic; it requires vitamin B for its development. Thus it may

be easily cultured upon the usual media and also upon synthetic media lacking vitamins. It grows well at laboratory temperatures.

Cultures should be made in tubes rather than petri dishes as the latter entail too much risk of exposure of worker to contamination.

Certain media have been recommended and discouraging the likelihood of contamination, especially for instance that advised at Stanford University—

Ammonium chloride	1 g
Sodium acetate	1 g
Potassium dihydrogen phosphate	0.8 g
Agar	1 g
Water	to make 100 ml

Autoclave 10 minutes at 15 lb pressure and before taking out add 0.04 g of copper sulphate per 100 g of medium. This prevents the development of most bacteria. Smith recommends this medium.

St. Wilhelm (1948) in view of the great tolerance of *C. muris* toward variation of pH (0.1 to 12.19) recommended the following—

Bacto tryptone	0 g
Sodium chloride	5 g
Bacto agar	20 g
Water	to make 1 000 ml

Place in 100 ml flask, autoclave and whilst the medium is still hot add 0.5 ml of normal hydrochloric acid then place in petri dishes. Inoculate copiously (0.5 to 0.7 ml of sputum per petri dish) and incubate at 37°C. From the beginning of growth which occurs in 3 to 4 days remove from the incubator to avoid desiccation.

Wilhelm also recommends the use of 1:2,000 methylene blue or acriflavine at the same dilution in the tryptone agar or together at the same concentration.

For microscopic examination submerge the petri dishes for 10 to 15 minutes in 10 per cent formalin; this does not alter the essential morphology.

It is also worth noting that techniques employed with sputum (acid fastness digestion) for the isolation of Koch's bacillus destroy *C. muris*.

Usually the morphology of the colonies and their microscopic examination will establish the identification of the fungi. However in a certain number of cases animal inoculation must be resorted to.

8 Inoculation

This can be made from pathological or cultural product. Two animals are normally employed—mice which reach the parasitic phase within 5 or 6 days in intraperitoneal injection and in any case die within 7 to 14 days and guinea pigs which after intratesticular injection develop an orchitis and the aspirated pus obtained towards the fifth day is rich in spherules filled with endospores.

Inoculated chlamydospores whilst undergoing transformation to sporangia may remain attached to one another thus yielding clusters of two or three sporangia which may present abnormal pictures of prolific development or of budding.

4 Cutaneous Tests and Serology

Substances develop in the culture media of *Coccidioides immitis* which when injected into the skin of patients and those recovered from coccidioidomycosis produce a reaction analogous to that which tuberculin gives in those individual made sensitive to Koch's bacillus. These active substances which are probably polypeptides resist autoclaving at 15 lb for 30 minutes. In 24 hours they partially dialyse through Cellophane membranes. The substance in question coccidioidin can be kept indefinitely but is liable to destruction by bacterial contamination.

For the preparation of coccidioidin C F Smith recommends culturing several strains of *C. immitis* for two months in the following medium—

Ammonium chloride	g
L asparagine	g
Potassium dihydrogen phosphate	1.31 g
Sodium citrate	0.90 g
Magnesium sulphate	1.5 g
Iron citrate	0.90 g
Glucose (quality cerolose)	10 g
Glycerine	ml
Water	to make 1 000 ml

Each of these substances is dissolved separately in distilled water and they are added in the above order to the asparagine. Complete by dissolving the glucose and glycerine and finally having brought up the volume to 1 litre sterilize at 115°C for 15 minutes. After culturing for two months the filtrate is diluted and 0.1 ml of a 1:1 000 dilution is used for testing. After 48 hours erythema and induration are apparent. Those who do not react are given another test in which the 1:1 000 is replaced by a 1:10 dilution.

Stewart and Kimura (1940) defined the coccidioidin unit (skin unit) as follows. A skin unit is the smallest quantity of coccidioidin which in a 0.1 cc dose produces in sensitive subject in 4 hours an erythema which lasts for 4 hours.

The cutaneous reaction to coccidioidin appears from the 1st day after the first symptom up to 10 days to 6 weeks after infection and persists for years so that when positive the only possible conclusion is that the patient had or has coccidioidomycosis. In serious cases of the disease the form the reaction may be negative.

Interpretation of a negative reaction may be demonstrated in the serum of patients and those who have been cured but usually the precipitum

lesion or within a month or two following (Fleming, H. and Lusk, 1918).

Complement fixation studies employing coccidioidin as antigen give doubtful or poorly marked results with the benign forms but rise of titre indicates an aggravation of the condition.

Study of blood sedimentation which increases during the period of invasion and that of dissemination may be of value. In three out of four of the cases of pulmonary involvement with cavitation there is, on the contrary, a normal sedimentation rate.

Willet and Werns have not detected the primary form at 10 per cent of eosinophils during the first week.

Coccidioidomycosis in Animals

Animals are apparently not subject to the primary form of the disease. The granulomatous form is the one encountered and more often than not diagnosis is made at the post mortem.

Except for rodents the greatest number of lesions have been described for bovines; the first was reported by Ciltner (1918) at San Diego, California. All the sick animals come from California, Arizona and New Mexico. Lesions in the bronchial ganglia or mediastinum or more rarely pulmonary nodules are found on killing the animal. Incubation period is less than that of the human disease.

Microscopic lesions are those of tuberculosis but the presence of spherules establishes the diagnosis. Spherules surrounded by eosinophilic cells comparable with a tinomyces reaction have been found especially in cattle.

According to Smith (1948) a case of coccidioidomycosis has been found in a gorilla (*Gorilla beringii*) and one in an American possum (*Didelphis virginiana*) at the zoo at San Diego, California.

In the dog coccidioidomycosis resembles the human primary form more closely than the very chronic form in cattle. In the three cases described nodules were found in the lung, liver, spleen, kidneys and brain. Two of these dogs lived or had lived in endemic zones. The third case is more curious (Phummar 1941; Radmore 1941) and is of a male setter from Canada which had been mated with a female from California. After having shown disturbances of the central nervous system the dog was accidentally killed. Autopsy revealed pulmonary and cerebral lesions of a tuberculous character but in which the characteristic spherules were found.

With the exception of rodents (see note following) animals do not appear to play any role in the spread or maintenance of coccidioidomycosis.

Taxonomy

It is generally agreed with Simmons that *Coccidioides immitis* is a phycomycete. However, Hartmann (1911) and later independently Langeron (1929) were the first to suggest this. The prefix if accepted in the form

CHAPTER VIII

Mycoses caused by Dermatophytes

Definition

The Dermatophytes form a group of pathogenic fungi which by a marked affinity for keratin attacking skin hair and nails. The word Dermatophyte which now has a precise meaning, was formerly used in a much wider sense. It denoted any member of the plant kingdom living upon the skin as a saprophyte or a parasite and in this sense certain bacteria were considered to be Dermatophytic (cf. Sabouraud in *Dermatologie Pratique* art. Dermatophytes. Douin Paris 1900). It is impossible to say who first gave to the word Dermatophyte its present restricted meaning. It is to be noted that it covers only the fungi referred to the Cymnoascetes by Matsuoka and Dierckx and grouped in the genera *Trichomyces*, *Sabouraudia*, *Trichophyton*, *Langeronia* and *Epidermophyton*.

The greatest confusion prevail in the dermatological nomenclature in denoting diseases caused by dermatophytes whether in France or elsewhere. We feel that in the French literature the word *Dermatophyte* used for long, but not precisely ought to designate disease caused by Dermatophyte. The word ringworm is applied by custom to a disease of the scalp and glabrous skin but it is difficult to apply it to *Trichophyton marginatum* and to mycoses of hand and feet caused by dermatophytes. The term *Dermatomycosis* has two broad meanings for it may designate all skin mycoses whatever the parasite. The term *Dermatophytosis* has mainly been used for dermatophyte diseases of hand and feet.

It is not here proposed to suppress term established (1) with a synonym *eczema marginatum herp. irritatus* Athlete foot. The phrase is merely for a precise term denoting all dermatophytic lesions which could be used thus: Dermatophytosis (dermatophytosis) of the scalp or *Dermatophytosis capitis* of the glans skin or *Dermatophytosis corporis* of the nails or *Dermatophytosis unguium* of the feet or *Dermatophytosis pedis* of the inguinal region or *Dermatophytosis inguinalis*. Mycotomycosis should be replaced by dermatophytic mycosis or dermatophytomycosis of the nails especially as it is probable that if the mycotic mycoses are ruled by dermatophyt

Historical

The history of ringworm goes back to the most remote time and precedes that of the Dermatophytes. There is an account in the *Procès de Teignes* by Sabouraud.

Dermatophytes have been known for more than a century, but their detailed study dates from the masterly work of Sabouraud. His recent work has been a stimulus to criticism here and the effort of his student, even in his opponent, has served only to extend its value.

In 1837 whilst studying the cutaneous fungus *Trichophyton* he noted that it was made up of mould filament, but did not connect the mould with the disease. In 1839 Schönlein of Zürich showed the connection between the disease and the plant kingdom.

However it was Gruby a Hungarian Jew, exiled in Paris who was the pioneer. From 1840 to 1841 he discovered successively the fungus parasite that of thrush, the first trichomycosis in the frog, then the parasite of microsporia, later then we call the ectothrix *Trichophyton* of the beard and finally the endothrix *Trichophyton* of *Ringworm* (Sabouraud 1936). Though he described very badly the symptoms of the diseases of which he discovered the parasite, in the matter of ringworm he committed only one not blameworthy error. Describing the polar lemon.

he makes his parasites grow upwards like hair when they all grow in the opposite direction it has downwards (Sabouraud 1936). Gruby's first work is paper in the *Bulletin Académie des Sciences* (1841) in which he describes fungus and shows that it can be destroyed by microscopic examination. In the same year he reported the inoculation of the fungus into man and animal and that he had at last succeeded in making it grow even on wood (Sabouraud 1910 p. 1).

In 1841 he discovered the trichophyton which Sabouraud later called *Trichophyton microsporum* and introduced the term porphygophytes (cryptogams of fungus), mentaglyphytes (cryptogams of the mentaglyph) and phytoglyphites (cryptogams of thrush). Yet in 1841 he described and named *Microsporum* which Sabouraud rediscovered in 1880. Quoting Gruby it is to be noted that the spelling of *microsporum* is not *microspora*. It is often written. I shall call these cryptogams *microsporum* because of the smallness of their spores and in order to commemorate in this new field of pathology the name of the famous academician who by his outstanding research on *Mycodermis* has contributed greatly in directing attention to the parasitic plant which destroys living animal tissues. I propose the name *Microsporum* for the plant organism which constitutes

1) Italicized Agostino Bassi found in 1835 the first known example of the disease caused by the parasite *Bombus olivaceus* (reduced form of *Bombus*) in the larvae of the *Bombus* species. Audouin described the *Bombus* in 1835. *Microsporum* is of course a name of the *Bombus* species. Cf. Agostino Bassi (1835) 24, 1, 16. Redfern (P.) 1930 (V) Agostino Bassi precursor of concepts in crop pathology. *Mycol. Pap.* 1930, 2, 1-4.

Porriga decalians: In 1804 Sabouraud once more found (rub) a work which had been forgotten for half a century and noticed that what he had described in 189 as *Trichophyton microsporum* (sic thus probably the spelling mistake) had fifty years earlier been described by Gruby under the name *Microsporum audouini*. He immediately rectified his mistake (1904) which was not merely one of bibliography but also one of observation because as Sabouraud himself wrote (1910 p. 8) *Trichophyton microsporum* the *Microsporum audouini* of Gruby was first described as inveterate and filling up the hair with small spores a mistake that Gruby did not make and which was corrected after reading his account. Gruby's series of mycological publications ended in 1844 with an investigation in which he described *Trichophyton endothrix* without naming it. Malin ten in 1845 introduced the neologism *Trichophyton* in a paper translated into German in 1848.

In 1846 Remak published the account of his attempts to grow and inoculate the favus fungus which he named *Achorion schenckii*. He stated that he had successfully inoculated himself with the fungus. Remak wrote Sabouraud (1910 pp. 66-7) obtained on an apple a beginning of vegetation of which he gave an unequivocal microscopic drawing. It was not however until 1886 that a series of pure cultures was obtained.

Within the space of four years Gruby had recognized the organism associated with favus and also *Microsporum* and *endothrix* and *ectothrix* *Trichophyton*. The clinical description which accompanied this mycological work was unfortunately so unprecise that confusion reigned for nearly half a century. Alopecia notably was attributed or not to *Microsporum audouini* and some claimed that there were versions of it with and others without *Microsporum*. The *Trichophyton* were no less resolved and Sabouraud was the first to be able to answer two key questions: the first put by Kral of Prague at the Tenth Congress of Medical Sciences at Berlin in 1890 on the plurality of the *achorions*; the other by Magnin in 1897 on the plurality of the *trichophyton*.

Sabouraud (1864-1939) (cf. Lançon 1975; Grigoriadis 1979) was stimulated to study ringworm by Hensner and under the influence of Roux and Duclaux he applied Pasteur's method. In addition he gained experience in the culture of Dermatophytes from Duclaux; he shortly after Crivitz was the first in France (1896) to obtain pure culture of *trichophyton* and favus; he also gained from the work of V. Roux (1897) who besides contributing interesting information on dermatophyte culture was the first to report the external spores which he distinguished from mycelial spores.

In his first work (1897) Sabouraud distinguished between the *trichophyton* associated with small spores and that associated with large spores. Further this work established that the latter form did not represent single species but groups of species. He ended the discussions between unicists and pluralist.

In 1894 Sabouraud published through Rueff in Paris *Les Trichophyties humaines* a book containing an atlas and in which he described many species of *Trichophyton* and distinguished clearly the parasitic organism *Microsporum audouinii* which until then he had called *Trichophyton microsporum*. At a recorder at the International Congress London in 1896 he demonstrated by means of cultures and microscopic preparations the plurality of the dermatophytes and established his findings.

Adamson in 1895 described the growth of dermatophytes at the level of the hair papilla going rise to Adamson fringe named after him.

In 1896 Bodin published his work on *teigne loupante* in the horse. This exerted a considerable influence on Sabouraud and from it emerged a clearer if still imperfect understanding of the pleomorphism of the dermatophytes which had until then been considered to be a species of symbiosis. In 1897-04 wrote Sabouraud (*Les Teignes* p. 84) "I was ignorant of the facts of pleomorphism known however to professional mycologists and I had interpreted these facts as a symbiosis in the same culture of two different entities but only one parasite. And many had accepted this theory of commensalism. Bodin in 1896 first brought symbiosis into question in connexion with microsporum of the horse. Together we appreciated the real nature of the facts of pleomorphism. Regarding symbiosis Bodin had indeed written (1896 p. 60) 'This polymorphism is an occurrence so widespread amongst lower fungi that the hypothesis of symbiosis is in some cases observed in trichophytic cultures seem to me if not doubtful at least warranting further mycological investigation. Already however some workers such as Hiral had deduced from this polymorphism that all the alleged trichophytic species described as entities were only reversible varieties of one type (Bodin 1896 p. 60).

Duciaux (Sabouraud 1936) was responsible for advising Sabouraud to call pleomorphism the *forme of mutation* which definitely alters the characters of a species.

His work had reached such a stage that Sabouraud owed it to himself to attempt to resolve therapeutic aspects of ringworm. After trying to find a hypothetical scurf toxin he found that thalium acetate used up to 1897 in the treatment of syphilis and eczema was an automatische means of depilation (Sabouraud 1936) but he soon discontinued its use particularly when in 1904 he discovered epilation by X-ray method which with Nourse he was able to apply *en masse* thanks to the X-ray meter of Sabouraud Nourse.

Whitfield in 1908 identified and referred to the dermatophytes what he called dermatophytic infections [*dermatophytiasis*] of hand and feet a group recognized by Hiral in 1890 and four years later he having infected himself accidentally with ringworm he discovered and used upon himself the ointment which bears his name. Ansell (1951) quotes him as saying "I then rubbed in an ointment of 5 per cent benzocaine and 3 per cent salicylic acids in soft paraffin and coconut oil for three days and it

disappeared. I venture therefore to recommend the use of this ointment in superficial tinea.

In 1910 Sabouraud published through Masson (Paris) that remarkable work *Les Teignes* which is really the third volume of a series devoted to diseases of the scalp.

In 1900, Plito and Verser isolated trichophytin and demonstrated the reaction to this substance by patients suffering from trichophytia. This was confirmed by Truffi (1914) but developed particularly by Bruno Bloch and his students (1928). The discovery by Margaret and Davis (1925) of the fluorescence under Wood's light of hair infected by *Microsporum* permitted of early diagnosis and easy supervision of the cure as well as the detection of contaminated objects. The classification of Otis and Langeron (1933) and that of Langeron and Malchevitch (1930) based on the cultural morphology of Dermatophytes and the use of natural media brought about the transfer of the bases of dermatophyte classification from parasitism to saprophytism. The principles were again used by Emmons in 1934 to support a classification which became of its simplicity is now probably the most widely employed. This survey would be incomplete without mention of the work of Grigorakis (1935) upon the classification of dermatophytes if only on account of the heated discussions which it evoked (cf. Sabouraud 1934-9).

Importance and Geographical Distribution

The dermatophyte infection (*dermatophytosis*) is a cosmopolitan disease found in men, women and children without any distinction of race or profession. This statement however requires some qualification.

In the first of worm affections of the scalp are usually of early childhood that arise spontaneously in infancy. This is not however invariable. If left untreated favus contracted during childhood persists for life. Scalp ringworm due to *Trichophyton violaceum* persists beyond puberty without difficulty without however having the harmfulity of favus. On the other hand rare cases have been reported of the occurrence of scalp ringworm in adults. Feldsher and Fennel (1918) have recently reported the appearance of a scalp ringworm with *Microsporum audouinii* on a woman of 46 having two children also infected with ringworm from the same dermatophyte. Reiff (1919) found a scalp ringworm caused by *Microsporum audouinii* upon a woman during pregnancy. This applies only to microsporums and common trichophytoses and not to squarred ringworms—kerions—that may be contracted at any age.

The reason for the spontaneous recovery from ringworm in infancy has long been a mystery but recent work by Rothman Smolysky and Shapiro (1935) has offered a plausible explanation. They have shown that fat obtained by extracting a lost hair with the solvent the growth of *Microsporum audouinii* at minimum concentration for 48 hours cent. the fungistatic power of the fat extracted from juveniles has been only

one fifth of this. The fungistatic activity is connected with the fatty acids while cholesterol, neutral fat and the unsaponifiable part have no action. From two batches of 1 kg of adult hair have been extracted 20.3 mg and 9.5 mg of active product respectively. The fatty acids which behave fungicides belong to the aliphatic series between C_{10} and C_{14} .

Sex. A curious and hitherto unexplained fact about scalp ringworm is its predominance in boys as compared with girls in the proportion of about three to one. This long known fact was rediscovered by Catane in North Africa (1933) and by Vanbreuseghem (1950) in Central Africa. In particular Catane observed for Muslim 8 per cent of favus in girls and 20 per cent in boys. In non-Muslims however the same writer found

per cent in boys and 4.1 per cent in girls—1.4 per cent *Trichophyton* species in boys and 3.7 per cent in girls—but these statistics are for a smaller number of cases. In subsequent work Catane (1938) found 84 boy scalp ring from ringworm as compared with 9 girls and noted on that occasion that in the Aures as in other regions of Algeria scalp ringworm has been observed with greater frequency in boys than in girls.

In the Belgian Congo Vanbreuseghem noted that 89 cases of ringworm due to *Trichophyton longirostre* were distributed among 64 boys and 25 girls. Again of 69 cases due to *Trichophyton ferrugineum* var. *album* 4 were in boys and 65 in girls. *Langermannia soudanica* affected 17 boys and 1 girl. On the other hand 10 boys and 9 girls had ringworm caused by *T. violaceum* and 26 boys and 27 girls by *T. globum*.

During the recent ringworm epidemic in the United States Schwartz, Pick, Botwinick, Leibovitz and Frauer (1949) found that in 490 cases caused by *M. audouinii* the ratio of boys to girls was 6:1. Schwartz, Rockwood and Chickel (1949) in the United States studying 989 cases of scalp ringworm over a period of six years (1941-46) noted that 61 per cent of the patients were boys and 39 per cent girls and that *Microsporum audouinii* attacked three times more boys than girls whereas *M. lanosum* attacked both sexes equally.

The predominance of ringworm in boys as compared with girls requires the support of important statistics but it appears to be well established. Some attempts have been made to explain these facts. It is said that boys get the hairdresser more often and are more exposed to contagion and again that the more abundant hair of girls protects the skin of the scalp against the implantation of dermatophytes. But these explanations hardly go far enough. Extraction of the hair fat of the hair of boys and girls by the technique of Rothman *et al.* (1945) would possibly be more revealing.

The capacity of certain species of dermatophytes to attack one sex in preference to the other is an insufficiently demonstrated possibility.

Dermatophytes of the feet appear to occur as frequently in men as in women. Schwartz (1947) noted this after examining 1393 men and 730 women of whom 28 per cent certainly had a mycosis. 34 per cent had

doubtful mycoses and 38 per cent were free from clinical sign. Athlete's foot however seems to acquire real importance with men more often than with women and no doubt this is the reason why the trichophyton reaction is more often positive with men than with women.

3 *Race* At first sight it seems that all races are equally receptive to all the dermatophytes. There are however certain facts which appear to indicate a predilection of certain races for certain dermatophytes or even races. Catani's observations in North Africa are instructive. He has observed that the proportions of favus and of trichophytia were different in the various populations of Oran. The white natives had 18.1 per cent of favus and 15.4 per cent of trichophytia, the black natives 9 per cent of favus and 15.4 per cent of trichophytia. He concluded (1935 p. 51) that "the proportion of white families contaminated by favus is thus higher than that of negroid families; these results agree with what we had observed in the study of the relationship between ringworm and race for all the subjects of the region."

It is not easy to explain these observed facts. It may indeed be a predisposition of certain races towards particular dermatophytes. Again it is possible that a dermatophyte may be incapable of establishing itself in a position already occupied by another, a hypothesis which may derive some support from the relative scarcity of mixed infections.

4 *Profession* Ringworm of animal origin is more usually to be found amongst those who work in contact with animals such as farmers, veterinary surgeons and soldiers who are particularly prone to lupus, spored *Trichophyton*. The greater frequency of favus in rural populations has been attributed by some to an animal origin of *Trichophyton schoenleinii* but this fact is far from proved.

Dermatophyte infections of the feet e.g. Athlete's foot are supposed to be more common among those who use swimming pools and shower baths. The occurrence of a greater proportion of Athlete's foot amongst those who indulge in violent exercise seems to be well established. Jell Keeney and Brojker (1915) found it on 80.9 per cent of 871 young military recruits. Montgomery and Casper (1914) claim that from 1940 to 1941 the American Navy had to send to hospital 14,069 recruits contaminated with dermatophytes, most of them localized in the inguinal fold and the feet. Muskatblitt (1933) in a comparison of 100 persons selected at random from a dispensary and 11 students found signs of pathological change between the toes of 80 per cent of these 11 subjects. This diagnosis was confirmed by culture or microscopic examination of 4.9 per cent of the students and 98 per cent of the others. Vain attempts have however been made to find dermatophytes in swimming pools and upon the cement and wood of shower baths. Williams (1933-34) made 1,400 unsuccessful cultures in a young men's college. Schwartz (1947) showed that in the laboratory it is possible to maintain *Trichophyton gypsum* on wood or cement but he

unable to isolate pathogenic fungi from the floor or cement of shower stalls after several hundreds of workmen had used them. However, Ambroseghem and Willvert (1911) isolated *Epidermophyton floccosum* from the sputum and urine of a patient with illa eczema marginatum. They showed that the receptacles used were infected mechanically by the patient without his knowledge. It is therefore probable that workmen carry fragments of pathogenic fungi when using a shower. The fact that they were not found does not prove that they have not been carried there.

It would seem that more than one activity must be involved in an environment which naturally favours the spread of the disease such activities being accompanied by a lack of local hygiene even though temporary as in the case of soldiers marching or fighting.

If dermatophyte infections (dermatophytosis) are cosmopolitan the same cannot be said for individual dermatophytes. It is clear that while certain dermatophytes are cosmopolitan others have a very limited geographical range; that every country and region possesses its particular spectrum of dermatophytes and that this spectrum is relatively stable. Thus *Trichophyton violaceum*, *T. rubrum*, *Epidermophyton floccosum* and *Microsporum audouinii* have a world-wide distribution while *Trichophyton ferrugineum*, *T. ajacis*, *Langroum boeckii* and *Trichophyton meckeri* seem especially to have a more limited geographical range. It is moreover clear that each region—this term is a vague one—has its own dermatophyte flora comprising a particular range of species with certain species predominant. Many examples may be given—

Sabouraud (1910) found 1500 strains of dermatophytes isolated and identified 147 *Microsporum* of human origin (13 *M. audouinii*) and 14 of animal origin (*M. canis*) making a total of 161 microsporida or approximately 3 per cent. The endothrix *Trichophyton*s numbered 10 (*T. crateriforme* 11 *T. acuminatum* 5 *T. violaceum* 90) or 44 per cent. There were 16 strains of neo endothrix, 90 microspores, 17 megaspores, 3 favus (*Achorion schoenleinii*) and 6 *Epidermophyton squale* (*floccosum*).

Forty years later (1950) Degos and Riabier reporting the dermatophytes isolated at the Saint Louis Hospital Paris analysed 811 cases of tinea as follows: microsporio ringworm 7 per cent, trichophytic ringworm 16.3 per cent, small spored ringworm 3 per cent, large spored ringworm 3.2 per cent, favus 40 per cent. Thus they compared with an analysis of 1127 cases obtained between 1930 and 1937: microsporio ringworm 51 per cent, trichophytic ringworm 29 per cent, small spored ringworm 1.8 per cent, large spored ringworm 0.6 per cent, favus 17.6 per cent. They noted that the trichophyton (half of them caused by *T. crateriforme* and the other half jointly by *T. acuminatum* and *T. violaceum* which is not very far from Sabouraud's results) are diminishing, but the microsporida in contrast are increasing (3 per cent in 1910, 51 per cent from 1930 to 1937, 57.5 per cent from 1948 to 1950) and that there is a marked increase in ringworm due to *Microsporum canis* (90 per

40 per cent actually 1 per cent in 1930 to 1937 and 9 per cent in the time of Sabouraud). It is to be noted that in Sabouraud's statistics referred to above the animal *Microsporum* due to *M. canis* (a *M. lanosum*) appear in only about 3 per cent of the cases. It is also apparent that there are 8 cases of microsporia caused by *M. ferrugineum* upon children from the Far East and that there is an epidemic involving the same species in Jews from Central Europe living at the Central Prison at Ghent, some of them having previously lived in Tashkent Prison, Turkistan.

The apparently unique statistic of these French workers emphasizes the persistence of the same species in the same places, the variations in percentage of certain species possible over a large number of years and the accidental introduction of certain exotic species.

During their studies of the ringworm of Eastern Scotland from 1946 to 1947 Hanner and Rogers (1948) noted that most of them were *Microsporum*, a total 813 cases caused by *M. audouinii* they found 8 due to *M. canis*, 5 to *T. crateriforme*, 1 to *T. sulfureum*, 1 to an unidentified ectothrix, *Trichophyton*, 1 to an unidentified ectothrix *Trichophyton* and 2 favus. In this result their almost 100 per cent microsporias contrast markedly with the 57.3 per cent of Saint Louis Hospital in 1940 and still more with the 3 per cent of Sabouraud in 1910.

Coudert and Doucet (1950) in a brief account of the ringworm of the Lyon region from 1946 to 1949 pointed out that of 14 cases were *Microsporum* (about 1 per cent) chiefly *M. audouinii*, but were misinterpreted ringworms (69 per cent) (*Trichophyton* 10 per cent, *T. album* 1 per cent, *T. roseum* 1), were favus (about 3 per cent) and 5 ectothrix *Trichophyton* all caused by *T. violaceum*. Thus even though the picture may have differed appreciably from the Parisian one the species involved were the same and no exotic species were introduced.

Late Coudert and Chancel (1947) have on the other hand noted the frequency with which *Trichophyton menthagrophytes* (3 cases out of 5) was found in the south east of France among dermatomycoses of animals and in the proportion is certainly not met with in Lyon where *Microsporum* and *Microspora* are active in the etiology of ringworm of animals only.

Popoff and Zocharskoff (1940) estimate that *T. violaceum* may be responsible for 60 per cent of ringworm in Bulgaria and in 1909 Nikolic reported that in 1 observation extending for four years he failed to find a single *Microsporum* and that most of the ringworm infection in humans are due to *T. violaceum*.

In Finland Iittala (1947) after studying 3 dermatophyte infections (*dermatophytes*) in man for four years found neither *Microsporum* nor *Microspora*. The ectothrix *Trichophyton* (14 strains) were represented only by *T. violaceum*. Other species isolated most frequently were *Trichophyton gypsum granulosum* (1 strain), *Epidermophyton floccosum* (floreus) (1 strain) and *Epidermophyton* (14 strains). An investigation in Finland and Hare (1940) verified the absence of microsporia and favus in an outbreak

T. schoenlei in Finland however *Achorion* (*Microsporum*) *gypseum* and 1 *gallinae* which we consider to be *Sabouraudia* were discovered.

Walker (1940) gave interesting information on dermatophytes isolated in Great Britain and Northern Ireland from June 1946 to September 1949. Out of a total of 473 strains the following were identified: 143 *Microsporum* *udoni* 544 *M. canis* 8 *M. gypseum* (1:199) strains of *Microsporum* about 50 per cent) 39 *Trichophyton* *albicans* 42 *T. schoenleii* *T. violaceum* 51 *T. dendroides* 53 *T. mentagrophytes* 11 *T. asteroides* 97 *T. rubrum* 16 unidentified *Trichophyton* *T. quinquiescens* *T. equinum* 4 *T. persicolor* 1 *Epidermophyton floccosum*.

Blunk (1941) gave some data upon the dermatophytes of Switzerland but in this comment must be taken into consideration that two thirds of his strain came from dermatophyte infection of the feet. He isolated *Sabouraudia* *udoni* 65 *M. canis* 5 *gypseum* 8 *Trichophyton* *schoenleii* 1 *T. flammula* 56 *T. rubrum* 78 (*T. asteroides* 9 (*T. griseolens* 3 (*T. asteroides* 9 *Epidermophyton floccosum*).

Lehman, Iphigene and Revonstam (1940) isolated out of 170 cases of scalp ringworm, derived in Texas from February 1946 to August 1948 11 *Microsporum* (65 per cent) and 5 *Trichophyton* (3 per cent) as follows: *Microsporum lanosum* (canis) 90 (53 per cent) *M. udoni* 19 (11 per cent) *M. fulvum* (*gypseum*) 6 (4 per cent) *T. mentagrophytes* 7 (14 per cent) *T. tonsurans* 4 (2 per cent) *T. violaceum* 9 (5 per cent).

Lewis, Hopper and Peck (1946) pointed out that during the ringworm epidemic of 1943 to 1944 in the United States 9 cases out of every 10 were caused by *Microsporum lanosum* whereas before the epidemic *M. udoni* and *M. lanosum* (canis) were found with equal frequency.

Burke and B. Warner (1949) observed in the United States that the following fungus is the cause of mycoses of the pharynx and skin on veterans of the second World War—

T. tonsurans 980 cases 10 out of 100 cultures 186 (18 per cent) *F. nigrum* isolated 118 *T. gypseum* (63 per cent) 9 *T. rubrum* (31 per cent) 4 *Epidermophyton floccosum* (4 per cent) 6 *Candida albicans* (3 per cent).

T. tonsurans *T. gypseum* was identified in 60 per cent of the cases. *T. tonsurans* from 79 points 8 *T. gypseum* 10 *T. rubrum* 1 *F. floccosum* 4 *C. albicans*.

T. tonsurans out of 31 points 1 *T. rubrum* (6.7 per cent). Out of 10 generalized dermatophyte infections (dermatophytosis) 7 *T. rubrum* was isolated 8 times from whites and *T. gypseum* once on white and once on a black.

In Algeria Catanei (1933) claimed to have observed 220 cases of favus caused by *Achorion schoenleii* and 528 cases of trichophytosis. From 84 per cent of 670 cultures of trichophytic hair he isolated *Trichophyton glabrum* and *T. violaceum*. 96 other *Trichophyton* were also isolated as follows (in order of frequency) *T. crateriforme* *T. fumigatum* *T. andersonii* *T. regularis* *T. cerebriforme* and *T. sulfuratum*. Further he described two new species namely *T. gourii* (Catanei 1933) and

species hitherto unknown in the region in question. They are caused either by real epidemics such as the recent one in the United States produced by *Microsporum adonis* or result from the operation of unknown factors as is apparent in the ringworm studies carried out in the Saint Louis Hospital during the last half century where the microsporia have acquired a prominence that they lacked in 19th century times.

It is a complete mystery why certain dermatophytic establish themselves in certain regions and not in others. Iitala and Harb (1930) commenting upon the absence of *Microsporum* from Finland suggested that this might be related to climate. They remarked that on two occasions they lost their cultures of *Microsporum* strains. The Finnish workers probably err at this fact. Many mycological centres keep strains of dermatophytes from various countries often very far away without any more difficulty in keeping them alive than is the case with their local strains.

The observations of Duval and Rivaller (1931) on the existence of a source of *Microsporum* (or *T.*) *ferugineum* in France supply an interesting contrast of viewpoint. Are zootic dermatophytes likely to spread from this source? We do not think so but it must be hoped that these French workers will continue to follow up their interesting observations.

Etiology

Human scalp ringworm diseases are usually transmitted in schools hence the name *trichomycosis*. During the recent epidemic caused by *Schoupsia* *adonis* American workers demonstrated the possibility of transmission via theatre seats and hand-drawn instruments.

Microsporum of animal origin differ clearly from the previous one in that an infected cat or dog is found to be their source. Duval and Gregory (1933) demonstrated the difficulty of diagnosis in the cat which can act as a carrier without any skin illness and the value of Wood light in the detection of infection in animals. Schoups observed 20 cases of circumscribed herpes caused by a family of Siamese cats and produced by *Microsporum felinum* (new).

The lesions and mycoses caused by small and large spored dermatophytes are mostly due to direct contact with an infected animal. Duval, Gregory and Bat (1934) observed 46 cases of suppurated ringworm caused by *T. albicans* and *T. gypsum*. All the patients were farmers or cattle merchants.

Human favus caused by *Trichophyton* (*Achorion*) *acharion* occurs mainly in poor families especially in rural areas. In France there are constant sources of favus (Jura, Somme, Cahados). Favus of animal origin arises from contact with infected animals e.g. game poultry.

Current work by Vanburenghien and Van Brussel has indicated that the dermatophytes can be cultivated on solid and starting the culture with infected hair leads to a pathogenic strain. It may well be that certain classical statements as to the etiology of the dermatophytes will require considerable modification in the very near future.

Hebra's herpes marginatus and the dermatophytes of the feet are probably favoured by warmth physical exercise and lack of hygiene even though temporary. The part played by hot water bath and swimming pools in their transmission is very debatable. Spring and summer and a generally hot climate favour their development. Passing from a hot climate to a cold one often results in the spontaneous regression of herpes clinical signs in the absence of treatment.

Keratolytic Power of Dermatophytes Parasitism of Hair

It has long been known that the dermatophytes are able to attack keratin. This fact is easily verified by noting in a *Microsporum* or *Trichophyton* preparation on a slide the hair broken a few millimetres from or close to the scalp or again by the examination of a nail attacked by *Trichophyton*.



FIG.

1) Microsporum of hair 1 1 mm (1) 1 of nail 1 mm (1)

Microsporum. The keratolytic power of dermatophytes may be demonstrated *in vitro* as well as *in vivo*. This was first demonstrated by Klotz in 1911 and later by Tinea (1920) and Tat (1933).

Despite prior observation in earlier times it was fully realized that dermatophytes growing on hair undergo a morphological development identical to that which characterizes them in culture media. This matter has recently (1943) been taken up by Van der Weerden who cultured a nail and varied ranges of dermatophytes on hair tested *in vitro*. He demonstrated that

1. The morphology of the fungus grown on hair is not different from that harvested from the parasite site.

Different methods may be involved in the fungus invasion of the hair *in vitro* namely either by the penetration of perforating hyphae or

by the progress of distention of the hair from the cortex toward the centre.

1 The breakdown of keratin can be observed not only upon human but also upon the most varied animal skin well on fathers.

4 The dermatophyte is the only fungus able to attack hair in this way.

This provides a means of facilitating the diagnosis of dermatophyte.

Vanbreuseghem thus established that there is a contrast between the development of dermatophyte in the parent and prophylactic states respectively.

Those who first studied the trichomycosis from Crabs onwards directed their efforts towards understanding the disposition of the parasite organisms within and around the hair. The descriptions of Sabouraud of the development of modern workers have simplified many matters which must have been complicated for the earlier workers. A instance of a controversy arose from Sabouraud's observation of a fungus growing from the spore sheath of *Microsporum*. Crabs who had previously noted it had given specific recognition to *Microsporum* alone whereas Sabouraud long regarded the organism in question as *Trichophyton microsporum* not knowing its distinctive morphology of structure.

Most of the current knowledge of the mechanism of the infection of the hair is due to the work of Sabouraud and to certain English workers of which that of Adamson (1905) is the most important. There is no better description of the mechanism of the penetration of the dermatophyte into the hair than that of Sabouraud given in a summarized form in 1908 (p. 100). Imagine one of these propagules cast upon healthy skin and about to develop there. The mother cell will surround itself with many branches diverging like the spokes of a wheel the resultant epidermis like an orbicular rod the rim of the wheel.

11 the region of rapid multiplication of the parasite. These filaments penetrating the corneal layer encounter the follicular orifice into which the corneal epidermis is collected follows by the thin descending into the follicle. However below the infundibulum in the corneal palmarium is maximum so the parasite deprived of its only medium for growth cannot advance further. If it is known that the hair which occupies the follicle and the hair is keratinized most of its length the neck of the polar bulb the parasite then lifts the cuticle of the hair (the cells of which are imbricated one upon another like tiles but in the proper direction that of the growth of the hair so that the nearest cell covers that of the one and thus presents a possible way of penetration to the descending dermatophyte) and penetrates it. If going in added it in depth with its filaments it goes down to the level where the hair is no longer keratinized beyond which it is no longer able to descend. (These terminal branches of the parasite make up what has been called Adamson's fringe which is the real zone of growth of the dermatophyte.) The bulb of the hair which is never imbricated does not react to the parasite and continues its function.

and so does the pilar papilla the hair therefore continues to grow as if it were not infected and so far as the hair is keratinized up to the level of the neck of the pilar bulb the parasite continues to invade it.

With this noteworthy account in mind the various types of parasite hair invasion will be described.

A. Forss. At the same time as it invades the hair the organism usually accumulates around itself an aggregation of mycelium included in



FIG. 1. Structure of hair by a photomicrograph showing the invasion of the hair from the periphery toward the centre.

the epidermis that it compresses beneath and around it. This is the favic scutula. The hair is invaded by mycelial filament which has divided into fairly long segments. Here and there secondary filaments from primary filament divide several times in succession to form the configuration known as the favic taurus or chandelier. These are polygonal aggregations composed of 8 to 1 very short mycelial fragments disposed in a manner which vaguely resembles that of the taurus.

B. Microsporum. The hair is surrounded by a sheath of mycelial filaments disposed without definite order in a mosaic. Within the hair itself are found mycelial filaments which are usually barely visible and descending toward the bulb. The spores result from the fragmentation of the mycelial filament into very small elements. The formation of the sheath of peripillary spores is certainly at first independent of the development of intrapillary mycelium. It is due to the subdivision of the mycelial

filament going down into the sulcy infundibulum between the hair and the epidermal cells. But Sabouraud admits (1910 p 197) that this sheath can be renewed and continued by the terminal ramifications of the intrapilary mycelium being able to graze the surface of the cuticle and end on there with a lot of spores more or less close to one another.

C. Trichophyton: The mycelial filaments invade the hair completely and reduce it to very small elements of square or oblong section becoming progressively rounded and forming regular chain. The spores are larger than those found in the sheath of the Microsporum. It was whilst studying the Trichophyton which produce this microsporic picture that Sabouraud coined the term *endothrix* of which the meaning has subsequently not always been fully appreciated; moreover it has been used both as noun and an adjective. In the first case it denotes dermatophytes which produce a pilous lesion similar to that just described. Sabouraud (*Les Teignes* 1910 p 263) is worth quoting. As early as 1893 I noticed (later than Gruby but without having read his text) that in the common *teigne tonsurée* the Trichophyton had *filles pore chainées et s'écaille avec elle* and for that reason I gave them the name Trichophyton *endothrix*. Thus the word *endothrix* should only be used to denote that particular condition of the hair when it is filled with spores.

D. Microles: The dermatophyte now called *Cirromyces* but which Sabouraud called microscidal Trichophyton invaded the hair by means of filaments which are usually barely visible also as in the case of the Microsporum they envelop the hair with a sheath of small spores in little chains. Sabouraud (*Le Teigne* 1910 p 273) recognized a pilous sheath comprising three elements—

- 1—very fine (3 to 4 μ) sheathed spores
- β —Pores of spores like strings of beads
- γ —Delicate (1 μ) non segmented mycelia float in the preparation in the form of fragments 10–20 μ long

The type of lesion thus constituted was called *ectothrix* by Sabouraud though this term is more usually applied to the form described below.

F. Ectothrix: In this parasitic type filament which may be reduced to large spores are found in the hair further they are surrounded by filament reduced into small chain of very large spores. Confusion is possible neither with the microscides nor with the microsporum and obviously not with the picture of *saïu* or *endothrix* trichophyton. Again quoting Sabouraud (*Le Teigne* 1910 p 263) I noticed whilst examining trichophytoses of the beard that the hair was *not only invaded but also surrounded by mycelial filament* and I named trichophytoses of this type trichophytoses *ectothrix*. This further quotation also explains much subsequent misunderstanding (note loc cit p 263). The definition

of ectothrix trichophyton has led to much confusion which I noted from the first especially at the London Congress. Most of the English workers believed that I considered the ectothrix to surround the hair without invading it and so Fox and Blaxal were able to write "Later we carehe appear to demonstrate that hair itself may be more profoundly implicated than Sabouraud at first observed and hence the term endo ect thrix (An inquiry etc p) And thus: how the term endo ectothrix whose definition is exactly the one I had given to the term ectothrix was created

Neo endothrix Sabouraud had thus named type of polar leucon characterized by the occurrence of spore in regular chain within the hair and with some filaments in an external position. The *endothrix trichophyton* he wrote (*J. Teigne* 1910 p 70) are certainly true endothrix for if a search is made amongst diseased hair one can invariably find some presenting the typical aspect of the real endothrix. But amongst these hairs a certain number one out of four are found which exhibit around the hair some striped filament between the hair and the follicle or attached to the surface of the hair. This is symptomatic of the commencement of a trichophyton only when the real endothrix trichophyton is involved this invading period is so short that its detection requires careful observation whereas when it concerns the trichophyton of which I speak it is prolonged to the point that it is difficult not to observe it.

Again Sabouraud wrote on p 763 "I had the various trichophytes may occur a four microspora type in human hair

- I True endothrix
- II Young endothrix (sp of) trichophyton
- III The ectothrix microspora
- IV The microspora

However in Vol II *Tratq Dermatologique* (1921) Sabouraud abandoned *neo ectothrix* and dealt only with ectothrix amongst which he distinguished besides the microspora certain parasites that appear to be of animal origin and that not only invade the hair after the manner of the endothrix type but also form around the hair a beath of irregular filament thus the ectothrix group and amongst the ectothrix type of animal origin there is a last group of peculiarities separated from the others because of the enormous size of their spore structure and which has been called microspora (p 105)

The following conclusion is clearly to be drawn from the foregoing type can be recognized in hair in isolated keratophyte as follows

- I The intrapil filamentous type represented by hair in the type of the agent of human favus namely *Trichophyton schoenleinii*

II The microspore type characterized by not very clear intrapolar filament and by a sheath of small spores in mosaic (Seborrheic) (Microsporum) aulon

III The true trichophyton or endothrix type the hair is filled with spores in regular rows or chains At the mature stage, if the infection there



FIG. 1

Endothrix I on wood I 2 clasp for J I (RV 1 16)
The II true endothrix I in clasp I I (optimal) even level I gl I I I on
the intrapolar myxos (400)

are no mycelial or spore bearing structures outside the hair. This type of lesion is produced by *Trichophyton tonsurans*.

IV In the ecto-endothrix type filaments are found within the hair and according to the particular dermatophyte responsible for the lesion a sheath of small or large spores is found outside the hair. In certain cases intrapolar spores may also be found. This fourth group appears to be confused and deserves complementary study. In particular such study should determine whether for a given species the parasitic type is constant or variable. In the case of the megasporous varying descriptions have undoubtedly been given by writers whose capacities for accurate observation are unquestioned.

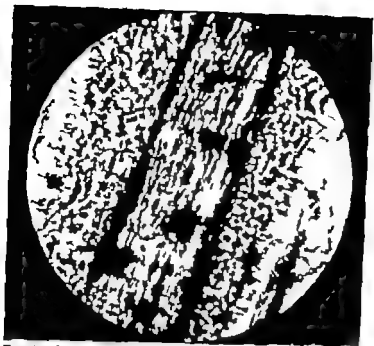


FIG 10 Hair of guinea pig parasitized by *Trichophyton* (strain 1 & 23) *Exothrix lessonae* polar follicle Mounted in chloral lactophenol



FIG 11 Hair of guinea pig parasitized by *Trichophyton* (strain 27) *Exothrix lessonae* polar follicle Mounted in chloral lactophenol

Classification of the Dermatophytes

Various classifications of the dermatophytes have been proposed. Three are given below: (i) that of Sabouraud the oldest based essentially upon the characteristic features of hair invasion by dermatophytes. Very much appreciated by clinical physicians especially dermatologists it still commands wide attention. (ii) that of Emmons is based on the characters of dermatophytes in culture. It is simple includes only the genera *Trichophyton*, *Microsporum* and *Epidermophyton* and is widely used in the English speaking world. and (iii) the classification of Langdon and Milchevitch slightly modified is based like that of Emmons upon the morphology of dermatophytes in culture and many European and South American report use it.

1. Sabouraud's Classification of the Dermatophytes

This is summarized in the table taken from Sabouraud *Les Teignes* and is essentially based upon the characteristics of the polar lesion. For example all dermatophytes which invade hair by surrounding it with a sheath of spores in the form of mosaics are classed among the microsporums. This can in any case be produced by microsporums of the human type—these are pure microsporums—or by microsporums of animal origin—the neo microsporums. This first group is the most coherent even though the morphology in culture of the microsporums of animal origin differs greatly from that of the microsporums of human origin these differences are however quantitative and not qualitative the microsporums of animal origin forming a very great number of macroconidial and microconidial types whereas these reproductive forms are rare if not absent in the human microsporums.

The second group that of the trichophytoms is more complex. Sabouraud divides them into endothrix and ectothrix. The endothrix types are in turn subdivided into pure endothrix and neo endothrix the ectothrix into microdes and megaspores. The significance of these various terms has already been explained. The coherence of this group is much less than that of the microsporums and if Sabouraud's basic feature of the classification i.e. the polar lesion is adhered to it is indeed hard to decide what resolves the grouping of these dermatophytes. The hair invaded by macrod or a megaspore differs completely from that invaded by a pure endothrix. The third group that of the chorions was based essentially on the formation of the scutula and comprised parasites of animal origin together with one human parasite namely *Achorion schoenleini*. It is to be recognized that towards the end of his life Sabouraud attached less importance to this small group. He had perhaps more than some of his students adhered to the idea of a botanical classification of the dermatophytes. Indeed he wrote (*Bouille Pratique Dermatologique* 1936 p. 120) 'Cases of favus of animal origin are rarely observed on man. Though scarce they have great interest for they will undoubtedly lead to a clarification of the subject especially in relation to the unity of

placed under new conditions where they can undergo full morphological development may produce very different form. Examination of certain members of the chorion group renders this very obvious. The type species of the group *Achorion achor* L.: has with good reason been classified amongst the trichophytes. *Achorion gyllenhammii* one of the agents causing mouse fur is also a trichophyte (Vanbreenseghem 1930). *Achorion gyllenhammii* and *A. gypseum* are dermatophytes which fall naturally into *Sabouraudia* (= *Microsporum*) of which they have all the characteristics. Sabouraud's opinion as to the systematic position of the latter has already been given. Putting other groups it will be apparent that *Trichophyton ferrugineum* which causes microsporic leprosy of hair should according to Sabouraud be placed with the microsporum and that *Longospora sordida* which causes endothrix ringworm should by the view of the same author be a *Trichophyton*. If we ever morphological studies which we have carried out on this pathogen have led us to regard it as the type of new genus (Vanbreenseghem 1930).

But perhaps the gravest objection to Sabouraud's classification: that by its very simplicity it runs counter to information on the morphology of dermatophytes in culture. It is too easy to imagine all the relevant data to be at hand once culture has been obtained from a pilar lesion of microsporic or endothrix type. This objection it must be said is not directed against the proponent of the classification. Sabouraud always aimed at perfect knowledge of the morphology of the dermatophytes which he described and left us the best possible figure of their cultural morphology.

In conclusion Sabouraud's classification takes many facts into account but not all. In our view it has a serious error. We consider that the classification of the dermatophytes must rest on the morphology in the media where they can develop fully and not on the morphology of the parasitic phase which because of convergent phenomena is misleading. For a full understanding it is to be added that if Sabouraud chose parasitic morphology as a basis upon which to establish his three great groups of dermatophytes because the morphology of the saprophytic phase best distinguishes the species.

2. Emmons's Classification of the Dermatophytes

The classification proposed by Emmons (1934) is based upon the morphology of the dermatophytes in culture. It is accepted by a considerable number of experts mostly Anglo-Saxon and we accept it success apart from the meticulous observations upon which it rests. Its simplicity and its respect for the essential feature of Sabouraud's nomenclature. This classification which Emmons proposed in the hope that following the lines of natural relationship it will stabilize the nomenclature of the dermatophytes if it is accepted. It goes back to three ancient genera (Emmons wrote at a time when bad impression had been created by the revolutionary nomenclature proposed by Ota and

Langeron 1923 and by Criborakis III) a mycological definition which up to a certain point seems to have been the intention of the creators of these names. This classification has the great merit of simplicity and therein lies the chief reason for its enormous success. However simplification is not everything and does not always lead to progress.

The following is a summary of Emmons's classification as he himself put it forward.

Dermatophytes These fungi are Hyphomycetes. They are usually white but in some species show some shade of yellow, pink, violet or brown. They reproduce by arthrospores, by chlamydospores, by single celled conidia which are subspherical or pear-shaped to clavate with broad bases, the base often being surrounded by a collar marking the point of attachment of the spore borne singly along the hyphae or in clusters on specialized conidiophores, aciculate or stipitate, sometimes forming chains of two or three spores and measuring 5 to 4 by 3 to 6 microns. They also reproduce by macroconidia which are clavate to spindle shaped or occasionally one celled but usually have from one to many cross walls and measure up to 40 by 160 microns in some species. They grow in the skin and its appendages where they are present only as mycelia or arthrospores.

Trichophyton Valmsten 1841. The type species is *T. tonsurans* Valmsten 1845. The mycelium is usually white but in some species it is yellow, pink, violet or brown. The organisms reproduce in culture principally by conidia. The macroconidia are clavate, thin walled and sometimes wanting.

Epidermophyton Sabouraud 1907 (not Lang 1879 or Vignun 1891). The type species is *Epidermophyton inguinale* Sabouraud 1907 which corresponds to *Epidermophyton floccosum* (Harz 1870) Langeron and Vukobrevitch. The mycelium is usually yellow, the organisms reproduce in culture by chlamydospores and by oval to egg-shaped smooth thick walled macroconidia.

Microsporum Gruby 1843. The type species is *M. audouinii* Gruby 1843. The mycelium is usually from white to brown, the organisms reproduce in culture principally by spindle shaped thick walled microconidia and clavate conidia. The former may be abortive or few in some species.

Following this series of short definitions certain dermatophytes are listed under the three genera adopted by Emmons. The genus *Trichophyton* includes all the small spored trichophyton (the microd) of Sabouraud under a single binomial *Trichophyton microsporum*.

Without going into all possible discussion of synonymy one is bound to wonder whether Emmons is right to include under a single genus both the classical and the microscidal trichophytons of the same author Sabouraud. We are against this for the following reasons—

(a) The microscids form a well defined biological group. They are above all agent of the suppurating ringworms represented by kerions and sycoms. It is conceded that this fact alone must not be used to assign the microscids to a separate group but it is fundamental taxonomic character.

(b) The microscid produce under cultural conditions morphological elements such as spirals, pectinate hyphae and antler hyphae. Even though these can appear in other genera and microsporums cultured in natural media can easily produce them it is nevertheless true to say that the microscids are the only ones to produce them with particular abundance on the usual media.

(c) The macroconidia (*aburres*) of microscids have particular characteristics—

(i) They are very generally spherical and not pyriform or laete like those of trichophytons.

(ii) They are disposed in clusters (*en grappe*). These are characteristic only of microscids and are absent from other dermatophytes though occasionally an early stage of their development may be observed in certain strains of *Trichophyton rubrum*.

(d) The macroconidia (*macroconidia*) of microscids are quite different from those of trichophytons. Emmons writes of the macroconidia of *Trichophyton megalophyte*. The macroconidium of *T. megalophyte* is clavate spore which may be as much as twice the diameter of the branch bearing it and it may be of nearly uniform diameter or somewhat inflated toward the distal end. It may have one or several septums. The walls are thin and hyaline. In some strains it is easy to find gradations between large clavate conidia and small macroconidia. The macroconidia suggest differentiated chlamydospores somewhat more than the conidia. In an occasional strain they are hardly more than hyphal tips which are differentiated by becoming several celled and are then detached bodily from the parent hypha. They vary greatly in size. Those which are one or twocelled may be 4 by 15 microns while the larger ones may reach 6 by 50 microns in size. In our view this description would apply to the macroconidia of *Trichophyton axys* Sabouraud (except for the microscids) and *axys* Langeron and Vlochevitch which are usually rare and poorly developed. *Trichophyton rubrum* is however an exception to the rule. This dermatophyte the study of which ought to be entirely repeated and the taxonomy of which is consequently in some doubt.

(cf. Vanbreuseghem 1949) is intermediate in morphology between the stenomyces or microoid and the trichophyton. It forms conidia (of size) of *Acladium* type pyriform but also often arranged in small clusters.



Fig. 1. *Microascus* (100 \times) (cf. 430).
 7. *Microascus* (100 \times) (cf. 430).
 8. *Microascus* (100 \times) (cf. 430).
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Further it may form filamentous crosswalled whorls which are long, thin and irregularly shaped.

Langeron and Miloché (1930) (488) wrote upon the morphology of the macroconidia of *Microascus*. The macroconidia of *Microascus* are markedly elongated and pointed at one end. The subarabid ha described their pyramidal form the walls attaining the extremity their of use a button shaped extremity the length at the end of the ability to form conidia (100 \times) a phenomenon the relation media.

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They are much better developed on our (natural) media than on classical media where they most often present an ill formed appearance either having the shape of short beaked macroconidia with the aspect of blastospores or resembling thick filament spindle shaped or drawn out telomorph telomeres.

We believe that Hammons has no justification on the basis of similarity of the morphology of the macroconidia for putting the trichophytes into the same genus as the asexual microconidia of *Sabouraudia*.

(2) There is yet further morphological difference between *Chromomyces* and *Trichophyton* arising from the asexual mycelium which presents the reproductive structures. In the trichophytes the macroconidia arise from asexual filaments as in the *Arthrospira* type. It is exceptional to find macroconidia in a number arising on a lateral branch at right angles to the asexual filament. On the other hand it is usual in the chromomycetes or microsporidia to encounter the lateral branches at right angles to the asexual filament and this branching system yields the Langeron-Croce formation bearing the clusters of macroconidia as characteristic of the species of this group. Again *T. rubrum* occupies an intermediate position between the chromomycetes and the trichophytes its asexual hyphae quite frequently forming Langeron-Croce which bear small clusters of macroconidia (cf. 10).

For the foregoing reasons we consider it undesirable to adopt the classification of the American mycologists. Though based on consideration which merit approval in its attempts at simplification it confuses certain features of dermatophyte morphology which are valuable in classification.

Finally it is suggested that adherents to Hammons classify the complex Fig. 14 of Croce's work (1918) on the so-called trichophyton with Fig. 5 of that of Croce and Muechling (1919) on a variety of *Trichophyton mentagrophytes*. It is impossible to disregard the considerable differences between the macroconidia and even the microconidia of the two species and the necessity in our view to assign these two dermatophytes to different genera *Trichophyton* and *Chromomyces* and not to one genus as is done by these authors.

3 Langeron, Milochévitch and Vanbreuseghem's Classification of the Dermatophytes

It is not proposed to deal separately with the classification of Langeron and Milochévitch as given by them in 1930 for it only differs from the present one in the inclusion by Vanbreuseghem of the genus *Langeron* in (1940). Certain new details were introduced in the first edition of *M. Langeron, Précis de Mycologie*. This classification like that of Hammons



From the ...
 The ...
 ... of ...



From the ...
 The ...
 ... of ...

is based upon the morphology of the dermatophytes in the saprophytic condition i.e. in culture.¹

(a) *Ctenomyces* Eadum 1890 Type *Ct. mentagrophytes* (Ch Robin 1877) This genus includes the microal trichophyton of Sabouraud and correspond to the single species that Fummons classifies amongst the trichophytom as *T. mentagrophyte*. It is characterized by microconidia

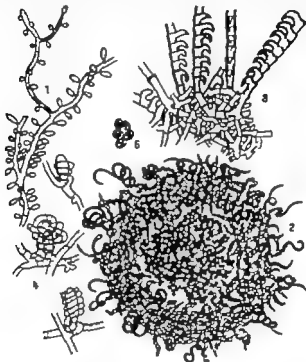


FIG. 1

Ct. myces verrucosus 1 Microconidial hyphae 2 Section of main hyphae with loosely woven intertangled hyphae and macroconidia 3 Mycelium showing perithecial formation (top shaped acroconidia with microconidia forming nodular bodies) 4 Group of acroconidia with macroconidia (After Eadum)

Large on and Mico be th ha d gnated Sabou and micro de by th name *Ctenomyces*. Th old g name wrote Lang on in the first edition of the *P d de Myc* p 81 as mnt d l to d gnate the ancient trichophyton micro d of Sab and because th y eth t fully th morphology of *Ctenomyces* fully d loped bushy con d al structures (m d) distaff shaped macro d p rth al ornamentation on m th form of lile sloped spirals borne p oosly filament with granula contents F ile p rth ra have not yet be ob rved but there th b gnarings of fleshy pe the mlar to tho of the *Gyrom* oal urrounded by tnd l and antileik filam te Now pres ly

longitudinal rows of long hyphae in the first and by distal beaked microconidia (for *S. aurea* *quercus*). Spiral and antler-like filament are found in cultures and are regarded as equivalent to the appendages of perithecia. In this genus the nodular bodies that usual to the commencement of a conium formation are cleared (Frimmon 1934). Aerial filamentous hyphae in this genus re-branched at right angles and terminate with the formation of Lorraine-Cross configurations. Dermatophytes of this genus attack hair forming a sheath of small spores in period of infection.

(1) *Sabouraudia* Ot. and Lancon 1923; Lancon and Melchior 1930; Type *S. aurea* (Cruikshank 1843). This genus corresponds to the genus *Microsporum* of Frimmon and to *Sabouraudia* genus *Microsporum* which are added next in species *S. gyp* and *S.*

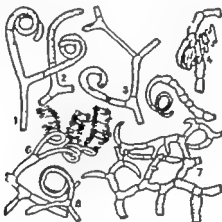


Fig. 1

1. Microconidium (spore) 2. Antler-like filament 3. Lorraine-Cross configuration 4. Beaked microconidium 5. Spiral filament 6. Nodular body 7. Aerial filamentous hyphae

S. aurea which *S. aurea* is placed in the genus *Sabouraudia* like the agent of human favus they are able to produce the appearance of scut. The *Sabouraudia* like species has microconidia of the *S. aurea* type and in pieces of animal origin very numerous macroconidia with lanceolate granular wall. Spirals appear only very occasionally upon ordinary media but are easily obtained on natural media. *Sabouraudia* species in attack form a sheath of small spores in the

(c) *Trichophyton* Malmsten 184 when Langeron and Mikoevitch 1930 see Ota and Langeron 193 Type *T. tonsurans* Malmsten 184. This group has very little homogeneity and though strictly it with respect we do not believe that it will retain its coherence. Vanbreuseghem (1930) has already detached from it a species necessitating in his view the erection of a new genus *Langeronia*. The morphology of *Trichophyton* cultures is very simple. Certain species develop conidia copiously like the *Acidinium* type along aerial hyphae. The macroconidia occur in exception

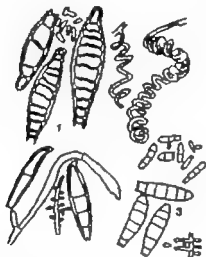


Fig. 11

Morphology of 1) *Trichophyton tonsurans* 2) *Trichophyton rubrum* 3) *Trichophyton mentagrophytes* 4) *Trichophyton violaceum* 5) *Trichophyton schoenleinii* (after V. Langeron)

ally cigar or sausage shaped with smooth and thin wall having blunt extremities the base being slightly larger than the filament from which it arises. Those species with glabrous colonies only except rarely form conidia. Spirals are not seen on ordinary media and only rarely appear upon natural media. Certain species (*T. tonsurans*, *T. rubrum*, *T. violaceum*, *T. rubrum*) produce a keratinolytic endotoxin which is characterized by large spores arranged in regular files in the interior of the hair. *Trichophyton ferrugineum* produces a microsporopollenin-like substance caused by the *Sabouraudia* species. *T. quinquemaculatum* causes the spontaneous appearance of scutula in mouse and man by inducing the hair unaffected. *T. schoenleinii* is the agent of human favus causing scutula in the hair which it does not break the appearance of filamentous reduced to arthrospores. The megaspored filamentous form and endotoxin keratinolytic with large spores in small hairs around the hair and certain species (*T. megnini* or *T. roseum* of Sabouraud and *T. rufum*)

produce an endo ectothrix lesion characterized by large intra and extracellular spores.

Essentially the genus is extremely heterogeneous and will undoubtedly be broken up. Various attempts have been made to establish subgenera: *Endotrichophyton* Langeron 1911, *Erytrophyton* Saboraud 1928 and *Megalosporosa* Saboraud 1928.

(d) *Langeronia* Vanbreuseghem 1930. Type: *L. soudanensis* (Joyeux 1911) Vanbreuseghem. This genus is represented by a single species. It

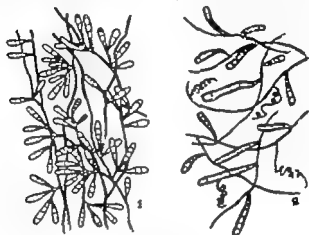


FIG. 6

Morphological limit of *E. dermatophytos* (f. sp.) after the original genus of Langeron (1870) and the reduced type of Langeron and Vukobrevitch (1930). The macroconidia are on the left and the microconidia are on the right.

soudanensis corresponding to the older *Trichophyton soudanense* of Joyeux. The vegetative mycelium with quite short articulations has marked tendency to form lateral branches growing opposite to the general direction of growth. Upon these primary lateral branches arise secondary lateral whorls with the same general characters of the primary ones. The reproductive spores are mostly arthrospores which are easily detached and may give rise in preparations to impressions of false branches. The true conidia are rare and arranged in the aciculate manner. The chlamydo spores which may be very abundant in old cultures are intercalary, terminal or lateral. Neither tendrils nor macroconidia are found.

(e) *Epidermophyton* Lang 1870 Ota and Langeron 1913. Type: *E. floccosum* Harz 1870. This genus is only represented by a single species. The reproductive forms are reduced to very numerous club shaped macroconidia often bunched like bananas. Langeron and Vukobrevitch have described tendrils in certain cultures but these organs are very rare. *E. floccosum* does not attack hair.

THE PRINCIPAL DERMATOPHYTES

I. Genus *Ctenomyces*

These comprise two groups—

- A. Cypripedium group with chalky colonies due to an enormous accumulation of microconidia—

<i>Ctenomyces mentagrophytes</i>	Ch Robin 1883
<i>Ctenomyces asteroides</i>	Sabouraud 1909
<i>Ctenomyces granulatus</i>	Sabouraud 1905
<i>Ctenomyces percoloratus</i>	Sabouraud 1910
<i>Ctenomyces interdigitalis</i>	Priestley 1911

- B. Nactus group with downy colonies—

<i>Ctenomyces radicans</i>	Sabouraud 1909
<i>Ctenomyces denticulatus</i>	Sabouraud 1910

II. Genus *Sabouraudia*

<i>Sabouraudia nudonini</i>	Cruby 1843
<i>Sabouraudia gypse</i>	Bodin 1907
= <i>S. fulva</i>	Urbancu 1907
<i>Sabouraudia canis</i>	Bodin 1907
= <i>S. felina</i>	Fox and Blaxter 1908
= <i>S. lanuginosa</i>	Sabouraud 1907
<i>Sabouraudia gallina</i>	Morgan 1881
<i>Sabouraudia elongata</i>	Vanbetuysen 1907
<i>Sabouraudia rivalis</i>	Vanbetuysen 1907

III. Genus *Trichophyton*

<i>Trichophyton tonsurans</i>	Miles 1841
= <i>T. crateriforme</i>	Sabouraud 1907
<i>Trichophyton rubrum</i>	Blanchard 1897
= <i>T. acuminatum</i>	Bodin 1907
<i>Trichophyton sulfuratum</i>	Fox 1904
<i>Trichophyton concentricum</i>	Blanchard 1897
= <i>Endodermophyton concentricum</i>	Castellani 1911
= <i>T. indicum</i>	Castellani 1911
= <i>T. tropical</i>	Castellani 1914
= <i>T. rugosum</i>	de Foa 1907
<i>Trichophyton quinquangulum</i>	Löffler 1900
<i>Trichophyton violaceum</i>	Bodin 1907
= <i>T. glabrum</i>	Bodin 1910
<i>Trichophyton violaceum</i>	Castellani 1909
= <i>Endodermophyton violaceum</i>	Castellani 1909
= <i>T. purpureum</i>	Bang 1910
= <i>T. rubrum</i>	Priestley 1907
= <i>T. lilacolor</i>	de Malashin 1907

- = *T. pluriconforme* MacCarthy 19
- = *T. laevigatum* MacCarthy 19
- = *T. coccineum* Y Kato 1938
- = *T. laevigatum* Kawasaka 1941
- = *T. A* Hodges 1941
- = *T. B* Hodges 1941
- = *T. salmoneum* de V Ho 1941
- = *T. rubrum* var III F ju 1941
- = *F. peract* Cast Han 1940
- = *T. lanuginosum* Fujii 1931
- = *T. spodi* Hat 1946
- = *T. arcolat* m Nelson 1949

Trichophyton ferrugineum (Ota 1941)

- = *Microsporum ferrugineum* Ota 1941

Trichophyton schoenleinii Lebert 1843

- = *Achorion schoenleinii*

Trichophyton album Sabouraud 1909

Trichophyton discoides Sabouraud 1909

Trichophyton magn Blanchard 189

- = *T. roseum* Bodin 1901
- = *T. roseum* Sabouraud 190

Note: It is necessary to note that the following forms are not species those described in 1938 by Langeron and Brumpt the names *T. m. forbesi*, *T. peract*, *T. truncat*, *T. deliens*, *T. iniae*

IV Genus *Langeronia*

Langeronia sordida (J Guet 191)

- = *Trichophyton sordida* J Guet 191

V Genus *Epidermophyton*

Epidermophyton floccosum Hara 1870

- = *E. cruris* Castellani 190
- = *E. inguinale* Sabouraud 1907
- = *E. clypeiform* MacCarthy 19

Microscopic Morphology of the Dermatophytes

It is virtually impossible to give a description of the dermatophytes in a work not devoted entirely to them. Others however have attempted this and profitable reference may be made to Brumpt, *Revue de Parasitologie* and Dodge, *Medical Mycology*. In identifying dermatophytes it is advisable to consult the earliest description if there is any doubt as to the identity of particular strain isolated. It is also advisable to try to obtain fresh strain of the species in question better still if possible to send strains for confirmation to the author of the species or to consult a mycologist with great experience of their pathogenicity. Unfortunately such authorities are extremely rare. Research workers who

specialize in study of dermatophytes are usually well acquainted with the species in their region and as a general rule hardly so with those of other regions. We have a certain experience in the study of the dermatophyte of Central Africa and have isolated a great many strains of *Trichophyton ferrugineum* of *Langeronia voudanensis* and of *Sabouraudites langeroni*. We must confess that not a single week passes without our experiencing some difficulty in classifying one of them. It is easy to imagine the difficulties in the way of anyone who only occasionally meets these species.

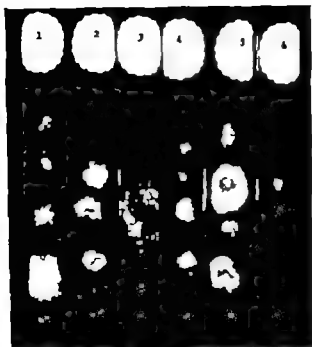
The identification of dermatophytes is rendered more difficult mainly for four reasons.

1. There are several systems of classification in existence. This is neither a fundamental difficulty nor in fact very important nor does it apply solely to dermatophytes; it is for instance equally true of mycology, helminthology or entomology. Having made an adequate study of the microscopic morphology of a dermatophytic culture and of the place to which it produces—or does not produce—one would only exceptionally be unable to deal with it under the system of classification adopted.

The many synonyms constitute a second obstacle to a simple classification. Ota and Kawamura had by 1913 enumerated 11 synonyms for *T. rubrum* alone.

3. Polymorphism in dermatophytes is a third difficulty. Thus polymorphism may appear in primary or sub culture. An atypical strain of a well known dermatophyte may thus easily be regarded as a new species. Vanbreuseghem (1930) worked with 130 strains of *T. ferrugineum* isolated in the Belgian Congo and was able to distinguish a white variety besides the classical form; further he demonstrated that each of these two varieties yielded four types of colony capable of isolation either simultaneously or separately from the same pathological product and of transformation from one type into another. In view of the fact that one of these types was a dense colony and another was a colony which resembled that of *Trichophyton schoenleii* it must be conceded that the identification of some dermatophytes is indeed particularly difficult.

4. Pleomorphism of dermatophytes is a precise, reversible transformation of the initial strain which becomes progressively less readily washed down much up fine sterile mycelial filament. The transformation is so radical that Sabouraud who first observed and described it long considered it to be a contamination or a bacteriological phenomenon of commensalism. The nature of pleomorphism is unknown; it is however known that its appearance is favoured by the use of sugar media and retarded by subculturing in non-sugary or conversely in media



P 1

1 Primary culture of *T. ferrug* strain R1 1036 on per cent glucose medium aged 39 days. Upper 3 colonies serpiginous and the lowermost on downy.

Primary culture of *T. ferrug* strain R1 1184 aged 31 days on per cent glucose medium. Top 1 bottom serpiginous cerebroid 1th row and retrograde down as the last being exactly of the *A. chrys* and the type.

2 *T. ferrug* strain R1 1036 isolated 1 month ago and subcultured in 1 ml. Tl. colonies on per cent glucose medium 3 days old. It is of the 1th row and 1th column precisely same in morphology as of *T. ferrug* isolated by Ota.

4 *T. ferrug* strain primary culture of strain R1 1189 aged 30 days on per cent glucose medium. From top 1 bottom the first and second colonies are of the serpiginous type the third is a very thin fourth leathery and serpiginous.

T. ferrug strain primary culture of strain R1 1189 on per cent glucose medium aged 31 days. At the same colonies of the 1th row type slightly lower 1th periphery. At the bottom cerebroid colonies.

6 *T. ferrug* strain primary culture of strain R1 1180 on per cent glucose medium aged 31 days. Colony of the serpiginous type with numerous satellite colonies.

(Photographs by H. Bauder)

Raising the temperature to 37°C also stimulates pleomorphism. Certain workers including Sabouraud maintain that only some species of dermatophytes undergo pleomorphism. Whatever the condition *Epidermophyton floccosum* rapidly undergoes pleomorphism. Other workers including the present author believe that all dermatophyte species must undergo pleomorphism; the phenomenon is not always so complete as in the examples described by Sabouraud and the whitening may not be so long nor so abundant but its occurrence seems indisputable. The rapidity of the pleomorphic transformation varies not only with the species but also with the strain. Pleomorphism unfortunately overtakes most cultures in culture collections. Undoubtedly these mycological centres are able to renew their strains and to supply a fresh typical sample to whoever requests a certain dermatophyte species, but if some years have elapsed since the isolation of the required strain one would receive only what the laboratory still retains as remnants not at all resembling the original strain.

Different classifications, confused nomenclature, polymorphism and pleomorphism such are the major obstacles to the research worker. Is there any remedy? We see only one, perhaps difficult to apply, but which we believe would result in a clarification of our ideas in a confused field and a quick reduction of multiple synonymy. Those who isolate ringworm fungi should send their strains—or preferably the product, such as a hair nail scale from which they have isolated the dermatophytes—not to one laboratory, but to five or ten all over the world and appointed by an authority on account of their knowledge of the dermatophyte flora of the region in which they work. Each laboratory would then carry out an independent identification the result of which would be communicated to the research worker either directly or through a central clearing office. Some light would frequently enough emerge from the nebulous mist. Though a harbour no ill wind as to the difficulty of applying this remedy we believe it to be the only effective one.

Microscopic Morphology of the Dermatophytes

The morphology of dermatophyte in culture will here be considered whether studied from the seed out fragment in double strength liquid from culture on solid or in hanging drops or in hair or skin.

The following points are important—

- 1 The vegetative mycelium
The aerial mycelium
- 2 The reproductive structure macroconidia (adult) macroconidia (fusiform) chlamydospore
- 3 Ornamented structures spiral (perforated) hyphae
hyphae
Nodular mycelium

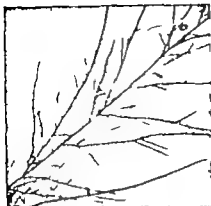


FIG. 2. *C. m. l.* (re 101)
 11 days aged (140)



FIG. 3. Same as FIG. 2 but t
 centre (10)



FIG. 4. Same as FIG. 2
 11 days aged (140)



FIG. 5. Same as FIG. 4 but t
 centre (10)



FIG. 6. *T. l. p. p.* at root
 11 days aged (140)

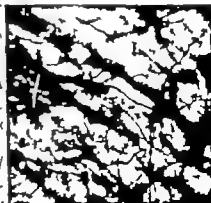


FIG. 7. Same as FIG. 6 but t
 centre (10)

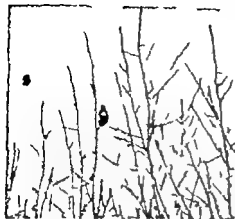


FIG 28 *Podernopsis flexuosa*
Blade culture aged 1 day. Peripheral
(x 140)

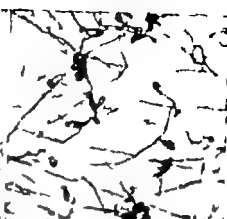


FIG 29 Same as FIG 28. Peripheral
(x 10)

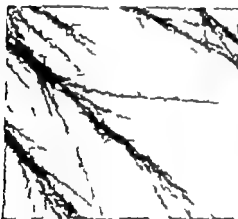


FIG 30 *Laciperna poul* blade culture aged 8 days. Peripheral
(x 140)

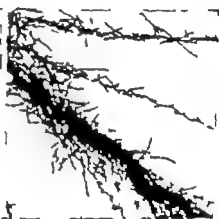


FIG 31 Same as FIG 30. Peripheral
centre (x 10)

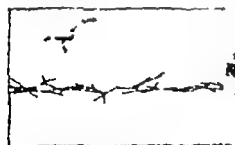


FIG 32 *Laciperna poul* blade culture aged 8 days. Detail of
branched blade net from peripheral
(x 800). Inset: Enlarged view of
pores forming image of microconidia
(x 800)

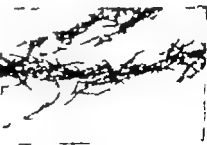


FIG 33 *Laciperna poul* blade culture aged 8 days. Detail of
branched blade net from peripheral
centre (x 10)

The Vegetative Mycelium

The vegetative hyphae appear first in culture. Whatever the origin of the culture from pathological products—nail, hair, scales—or fragments of culture of macroconidia, microconidia, chlamydospores or of mycelium, the germination of these elements always results in the formation of mycelial tube of 2 to 4 μ directed towards the periphery of the surface of the culture medium and which, after travelling for 20 to 40 μ septates. The parts of the hyphae enclosed between two septa are called segments. Once formed, the segment does not change, but it often gives rise to secondary hyphae which are mostly born immediately behind the distal septum directed towards the periphery and make a more or less obtuse angle with the hyphae from which arose.

It happens occasionally that certain secondary hyphae instead of growing towards the periphery go backward. This is exceptional except in the case of the genus *Leptothyria* where it is the rule.

The segment of the vegetative hyphae may swell at a short distance from the distal septum. This type of hyphae which is more frequent in certain species than in others is bordered by a thin line on the narrow side. In yet other cases the vegetative hyphae bend in arcs of circles and protuberances appear on the concave side of each arc; these protuberances have given the name of peritrichous hyphae. They are most clearly seen in the species *Sclerotinia fructicola* (Van der Schueren 1911).

The segments of the vegetative mycelium may fragment by the formation of transverse partitions; these fragments are arthrospores. When liberated from their parent segment, the arthrospores may give rise to macroconidia whose origin is quite different.

In certain dermatophytes, quite long fragments of a segment may become isolated, divide into cells and present the appearance of macroconidia. This is particularly clear in certain strains of *Trichophyton rubrum*. Many workers relate these to the macroconidia which arise on the aerial mycelium. This is not to be accepted without reserve.

Upon immersing themselves into their support, the filaments of the vegetative mycelium may wind up into a spiral; this is clearer in culture sections. These spirals have nothing in common with the spirals appearing in cultures of *Cytospora* and care must be taken to avoid confusion.

The Aerial Mycelium

This is characteristic of downy or powdery cultures. It arises on the vegetative mycelium behind a distal septum and coils slightly about itself at its point of origin. It is thinner than the vegetative mycelium and becomes shortly segmented. It may terminate abruptly or alternately travel over long distances in a straight line. The aerial mycelium bears reproductive structures.

has already been sufficiently described. It is to be noted that the genus *Epidermophyton* which does not form macroconidia produces macroconidia in clusters like bunches of bananas. Those of *Salsomundula* are lanceolate with thick and granular wall. The *Trichophyton* have cigar-shaped macroconidia with fine walls. Those of the *Chromomyces* species have irregular wall with a large base and an obtuse extremity.

Chlamydospores arise upon vegetative or aerial mycelia. They are rounded cell of double contour which may be terminal, lateral or intercalary. They are sometimes chains especially in old cultures.

Ornamented Structures

In *Chromomyces* and sometimes in other genera when natural culture media are used there usually appear spirals (coiled hyphae with tight spirals) dikaryon or heterokaryon which arise from the end of thick and granular vegetative hyphae. Less frequently thick vegetative hyphae may terminate with hook or anchor-shaped hyphae. Dickerson and Crook (1937) have made a detailed and interesting study of these forms.

Modular Bodies

These have been described by Saksena and co-workers with *Chromomyces* (*Trichophyton*) *lactorum*. They are regarded as rudimentary sexual structures (Fennell). They are like microconidia but have dense protoplasmic content and are typically knotted. They develop in a similar fashion to other forms of reproduction. Saksena noted that in old culture they may grow by putting out erect filaments.

Pleomorphism

The morphism of dermatophytes has been defined (cf. p. 10) as an irreversible transformation of the individual strain which varies itself progressively with a whit downmark. The fine structure, metabolism and some of the conditions governing its appearance have been indicated. A little amplification is desirable.

Pleomorphism is irreversible. A number of workers have attempted to bring about the reversion of pleomorphic strain to their initial morphology. Acton and Dey (1934) claimed to have achieved this by growing pleomorphic strains on feathers. Lanzeron and Milchevitch (1937) showed that this apparent success was probably due to the fact that they had used incompletely pleomorphic strains. Cifuri and Pedacchi (1947) claim in certain cases to have observed a return to normal morphology by keeping pleomorphic strain upon natural media in the dark for

By growing pleomorphic strains of dermatophytes (*C. albicans*, *C. parvum* and *C. immitis*) upon the soil Vanbreuseghem and Van Brussel have demonstrated that it reverts to perfectly normal structures non-pleomorphic as in its reproductive form. If these have at one or several points applied to all dermatophytes the dogma of the irreversibility of pleomorphism will be undermined. The first account will be in the *C. R. Soc. Biol.* (1951).

three years Hruček (1936) failed to bring about reversion to the type culture by varying pH, oxygen tension or by any other means. He reported a curious and as yet unconfirmed phenomenon, i.e. that pleomorphic strains of *Achorion gypsum* kept at 37°C change into culture of the fusiform type, and when brought back to 22°C the cultures revert to their usual pleomorphic type.

Pleomorphic strains can be inoculated into animals (Langeron and Talice 1930) and into man (Hruček 1936). The re-cultures yield pleomorphic colonies. Langeron and Talice (1930) by inoculating the guinea pig with a pleomorphic strain of *Sabouraudia filiformis* observed that the pile-like lesion was composed solely of intrapilar filament. On the other hand they noticed that this inoculation immunized a guinea pig against a previous inoculation of a non-pleomorphic strain of the same dermatophyte.

Citaneš (1933) summarizes as follows an important experiment upon pleomorphic strains. 1. When the normal pile-lesion is of the endothrix type, that which the pleomorphic culture produces is characterized by a reduction of parasitism. The intrapilar element remains filamentous, the formation of arthrospores being reduced or completely suppressed.

When the normal pile-lesion is of the ectothrix microsporic, microfilid or megaspore type, it is apparent that the characteristic sheath is not longer formed around the hair in the manner produced experimentally by pleomorphic culture.

On the other hand the author likewise considers that the white dwarf is not the only pleomorphic form. He proves this conclusively by the fact that certain degenerate forms in culture in the hair identical with those produced by the classical pleomorphic form.

The Law of Specificity of Dermatophytes

Much has been written about this law defined by Sabouraud (Le 7^e p. 40) as 'the law of correspondence between the clinical type of lesion and the parasite causing it' and it has given rise to various discussions. However expressed in the following brief summary. The law of specificity of the dermatophytes states that the most highly differentiated dermatophytes usually produce a recognizable lesion (Sabouraud in Schöer, *Medic. Hockensack*) it is admirable by very much as a general principle. It is certain that a scalp microsporia (microsporia) is not derived from a trichophytia (trichophytia) that a lesion is generally caused by the species *T. tricolor* that a favus nutula is mostly caused by the species *T. schoenleinii* that *Trichophyton rubrum* more than any other dermatophyte is the cause of tinea and persistent infections of the glabrous skin that this same dermatophyte only exceptionally attacks the scalp that *T. tonsurans* (tinea imbricata) is caused by only one dermatophyte *T. concentricum* that *Epidermophyton marginatum* is mostly frequently caused by *Epidermophyton floccosum* *T. rubrum* or *T. tonsurans* that dermatophytes (animal origin) usually produce purulent

lesions where as those of human origin are generally dry. But this is a far case in y go *Sabouria diti audon* & ca: *Trichophyton solaceum* re not alw y responsible for the same lesions. *T. solaceum* for example usually produces a scalp ringworm but Majocchi in 1907 described it producing trichophytic granuloma and Teberno onboff in 1928 reported the occurrence of *T. solaceum* in bony cavity. Kaplan and Ra. loschek (1918) report la ca: infection cau d by *T. arhoe* l and could find only three more such case reported in the literature. Hadada, Marill and Mourru described case of generalized favus on 16 year old Muslim girl in whom in l ment f the lymph nodes f the right inguinal region had indicated a picture f Nicola Favre disease. Haemoculture in this case had been posit ve.

E idently if the law of specificity f the dermatophytes applies to good many cases oft n it does not. The cau of these reactions is uncertain in some cases it is due to a particular pathogenicity of certain strains in theret a y uticular receptivity on the part of certain patients.

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Dermatophyte symptomatology f which the reader can find excellent descriptions in almost any treatise on dermatophytes will not here be described at length. The following may profit ably be consulted. Vol II of the *Vous II Pratique Dermatologique* Paris Masson 1936. *A I Introduction to Medical Mycology* by Lewis and Hopper 1949 and *Manual of Clinical Mycology* 2nd Ed by Conant et al 1946.

1. Dermatophyte Infections of the Scalp or Ringworm Proper

(a) The microsporiasis (*microsporia*). These diseases occur before puberty mostly upon children f school age. The lesion appears as a rounded plaque f average diameter 4 to 6 cm at the level f which re found hairs broken 2 or 4 mm from the pilar orifice. The hairs re surrounded by a whit sheath as if they had been dipped into flour or fine sand after ha m been smeared with glue. The microsporiasis of animal origin are often accompanied by a slight suppuration. They re cured spontaneously at puberty.

(b) The trichophyte infections (*trichophytia*). These are also found on children of school age and are characterized by small irregular plaques upon which are found short broken hairs sometimes so short that they are red ced to black points intructing the pilar orifice. Their extraction is difficult and they are cured at puberty.

(c) Favus. This is acquired in infancy but may persist for life. Near to non broken but lustreless hairs scutula are usually found surrounding the pilar orifice. These ha e a sulphur yellow colour and emit mouse like odour. The lesions develop and leave deep scars which end in incurable baldness.

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known where those of human origin are generally by. But this is as far as one may go. *Seborrhoea trichodynia*. Some *Trichophyton violaceum* are not always responsible for the same lesions. *T. violaceum* for example usually produces a scalp ringworm but Majocchi in 1907 described it as producing trichophytic granuloma and T. Hermannoff in 1928 reported the occurrence of *T. violaceum* in a bony cavity. Kaplan and Rabinovitch (1936) reported a case of kerion caused by *T. violaceum* and could find only three more such cases reported in the literature. Hadida, Marill and Mourer described a case of generalized folliculitis on a 16 year old Tunisian girl in whom involvement of the lymph nodes of the right inguinal region had indicated a picture of Nicot's folliculitis. The Haemoculture in this case had been positive.

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(b) The trichophyte infection (*trichophytie*). These are also found on children of school age and are characterized by small irregular plaques upon which are found short broken hairs sometimes so short that they are reduced to black points obstructing the polar orifice. Their extraction is difficult and they are cured at puberty.

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(d) Kerions are rounded plaque more elevated, suppurative and attack the head at any age. The hair is spontaneously expelled from the



FIG. 3

Large pit of micro fur in the scalp

plum orifice which milium pus. Recovery is spontaneous. In kerions are usually caused by micro fur. The hairs may be broken or intact.

2. Dermatophyte Infections of the Beard

These are also generally called kerions or paronychia. They are produced by various dermatophytes of which the microspores are the most important. They are found in adult men and possess the same aspect as the kerions of the scalp. They cure spontaneously. It is to be noted that the microbial vesicles are especially localized beneath the nostril perforation.

3. Dermatophyte Infections of the Glabrous Skin

Upon the glabrous skin may be found circinate kerion the centre of which appears to be healthy whilst more elevated edges are vesicular and extend to the periphery. Neighbouring ring may fuse. These lesions commonly called herpe circinate or Saint Catherine wheel appear on adults as well as children. In our countries *S. can* seems to be the most frequent agent.

Under certain conditions *favus* may invade the glabrous skin and form scutulae for the scalp.

Kerion are frequently found on glabrous skin. They are caused by *Trichomyces* or *Microspora* *Trichomyces*. They are in contact with human



6 The Dermatophytes

Cutaneous or other lesions secondary to a pre-existing dermatophyte infection are named thus. They will be considered in the section on allergy.

Histopathology

There is little to be said on this subject for the dermatophytes are primarily diseases which affect the superficial keratinized layer of the epidermis. However the structure of favic acutula, the nature of favic alopecia, the trichophytic granulomas have evoked a great many studies which need not be considered here for they make no essential contribution to the problem of the dermatophytes.

Treatment

Dermatophyte therapy varies according to whether the regions affected are the scalp, the skin or the nails. The following account is a broad summary of the treatment of these three regions when subject to dermatophyte infections.

1. *Treatment of Scalp Ringworm*. As they are maintained by infection of the hair, the methods of treatment of scalp ringworm involve either depilation or alternatively destruction of the dermatophytes in the hair.

(a) Depilation

The first method ever to be used was mechanical removal of the hair by means of pincers or a wax cap. No longer used alone, this method is however used to improve depilation made by other means.

The second method is depilation by thallium acetate devised by Sabouraud in 1897 but soon afterwards abandoned by him because of its dangers and especially on account of the introduction of depilation by means of X rays. It has fallen into disrepute which is perhaps regrettable.

Thallium acetate administered *per os* induces hair fall after the seventh day. Fall is completed from the sixteenth to the eighteenth day and regrowth occurs after the third or fourth week. X-ray does not the thallium does not extend to the eyelids and the inner region of the eyebrows. Early regrowth (perhaps due to the fact that methylated thallium have a stimulating effect on regrowth of the hair (Cooper and Engman 1931) and also the danger of intoxication constitute the principal objections to its use. Hairs reappearing after the third or fourth week stand a greater chance of being contaminated by hairs which have not yet fallen (after X-ray treatment regrowth occurs only after the second month).

A recent method of treatment of scalp ringworm is the use of X-ray. The method is simple and safe. It is based on the fact that X-ray treatment of the scalp induces hair fall. The fall is completed after the seventh day and regrowth occurs after the third or fourth week. X-ray does not the thallium does not extend to the eyelids and the inner region of the eyebrows. Early regrowth (perhaps due to the fact that methylated thallium have a stimulating effect on regrowth of the hair (Cooper and Engman 1931) and also the danger of intoxication constitute the principal objections to its use. Hairs reappearing after the third or fourth week stand a greater chance of being contaminated by hairs which have not yet fallen (after X-ray treatment regrowth occurs only after the second month).

Toxic effect due to thallium can't manifest themselves by muscular and articular pain polymyositis and by a temporary albuminuria. This remedy cannot be administered after puberty because it causes glandular atrophy. The optimum dose varies from 8 to 80 mg per kilo of weight and the dose cannot be repeated before two months.

Thallium which can be isolated (Crookes 1861) from copper pyrites marcassite ore blende is widely distributed in the plant kingdom e.g. in chicory tobacco wine laurel root and birchwood. It is however usually obtained from lead chromates and furnace burning thalliferous pyrites. Though it first employed in the treatment of syphilis and cystitis it was soon abandoned because of its cumulative toxic effects.

According to Davidson (Reports of Brit.) it should be administered as follows: children are weighed naked for two days running and a complete physical examination and run test made. Chemically pure thallium acetate is a very fine powder which must be weighed twice and carefully placed in a labelled bottle. The correct dose—9 to 80 mg per kilo—is the lowest weight in kilos multiplied by 8 or 8. When ready to be taken the thallium acetate is dissolved in about 100 cc of sweetened water taking great care that the whole of the salt is dissolved. Children over two years old and those appearing to require a dose more than 100 mg should not be subjected to this treatment. Having taken the medicine the child is put to bed for 4 hours. The head is shaved and coated with tincture of iodine three times a day. After three days in hospital the child is sent home with two caps one to be worn while the other is washed. After 4 days the hair is washed twice a day with soap. According to these workers hair fall commences on the 6th day towards the thirteenth day and is complete from the twentieth to the twenty-fifth. Infected hairs tend to fall later than healthy ones and regrowth commences before all the hairs have fallen. The removal of the old hair must therefore be accomplished with great care and tincture of iodine must be conscientiously applied.

Though X-ray depilation is the best method the thallium acetate method has certain value. Details for it we have been given fully because well administered thallium acetate is less dangerous than bad X-ray application.

The X-ray technique cannot be acquired from book and when well applied it gives more certain results than thallium because hair fall occurs during the third or fourth week whilst the new growth does not appear before the second month. There is thus time to observe the child's head after hair fall and to remove any infected hairs which have not been eliminated. Remarkable results have been obtained at the Lailler school by skilful application of this method but the precautions to be taken for good results without danger can be learnt only by practical demonstration. Elsewhere disastrous results have been encountered such as definitive baldness or therapeutic failure resulting either from insufficient dosage or bad distribution of the radiation.

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X-ray treatment of scalp ringworm on the scalp. The following table shows the results of treatment of scalp ringworm by X-ray. The results are given in the following table.

Case	Age	Sex	Site of lesion	Duration of disease	Time of treatment	Result
1	12	M	Scalp	6 months	10 days	Complete cure
2	15	F	Scalp	3 months	10 days	Complete cure
3	18	M	Scalp	12 months	10 days	Complete cure
4	20	F	Scalp	18 months	10 days	Complete cure
5	22	M	Scalp	24 months	10 days	Complete cure

Toxic effect due to thallium acetate manifest themselves by muscular and articular pains, polyneuritis and by a temporary albuminuria. This remedy cannot be administered after puberty because it causes glandular atrophy. The optimum dose varies from 8 to 8 mg. per kilo of weight and the dose cannot be repeated before ten months.

Thallium which can be isolated (Crookes 1861) from copper pyrites, marcasite, zinc blende is widely distributed in the plant kingdom e.g. in rhubarb, tobacco, wine, beetroots and hickwood. It is however usually obtained from lead chambers and furnaces burning thalliferous pyrites. Though at first employed in the treatment of warts and cysts it is now abandoned because of its cumulative toxic effect.

According to D. and O. Gregory and Hart it should be administered as follows: children are weighed naked for two days running and a complete physical examination and urine test made. Chemically pure thallium acetate is a crystalline powder which must be weighed accurately and carefully placed in a labelled bottle. The correct dose—8 to 8 mg. per kilo—is the low weight in kilos multiplied by 9 or 8. When ready to be taken the thallium acetate is dissolved in about 100 c.c. of sweetened water to keep parent easy that the whole of the salt is dissolved. Children over twelve years old and all those appearing to require a dose more than 300 m. should not be subjected to this treatment. Half the dose of the medicine the child is put to bed for 4 hours. The head is shaved and oiled with tincture of iodine three times a day. After three days in hospital the child is sent home with two caps one to be worn while the other is washed. After 1 day the hair is washed twice a day with soap. According to these workers hair fall commences on the second day towards the thirteenth day and is complete from the twentieth to the twenty-fifth. Infected hairs tend to fall later than healthy ones and regrowth commences before all the hairs have fallen. The removal of the old hair must therefore be accomplished with great care and tincture of iodine must be conventionally applied.

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The X-ray technique cannot be acquired from books and when well applied it gives more certain results than thallium because hair fall occurs during the third or fourth week whilst the new growth does not appear before the second month. There is thus time to observe the child's head after hair fall and to remove any infected hairs which have not been eliminated. Remarkable results have been obtained at the Lailler school by skilful application of this method but the precautions to be taken for good result without danger can be learnt only by practical demonstration. Elsewhere disastrous results have been encountered such as definitive baldness or therapeutic failure resulting either from insufficient dosage or bad distribution of the radiation.

Depilation by thallium acetate or X rays is essential and should be reserved for scalp ringworm of human origin such as *Sabouraudia audouinii*. This matter will be further considered later. On the other hand ringworm of animal origin e.g. *S. canis* with their natural tendency to heal spontaneously would appear to be more suitably treated by one of the following method which expedite recovery.

(b) External Treatment of Scalp Ringworm

Though Sabouraud frequently denied that ringworm could be cured by the application of medicament because of the inaccessibility of the dermatophytes concerned many attempts have been made along these lines during the last few years. Enthusiastic reports have given the impression that scalp ringworm is now only a trivial therapeutic problem. However enthusiasm has often been far from general on account of frequent misinterpretation of successful result. Rivaller (1940) has noted recently that there are microsporria—even of human origin—which are spontaneously curable and Hligman and Anderson (1941) in an article noteworthy in more than one respect have reopened the whole question. From their experiences during a recent epidemic in the United States caused by *Microsporum audouinii* they concluded that—

1 Spontaneous cure occurs in a large number of people suffering from ringworm

2 In a large number of cases the infection is accompanied by an inflammatory reaction if this subsides healing follows

3 So called cures resulting from the local application of fungicides are really spontaneous cures. This opinion was based on the observation of 199 cases in which the same percentage of cures (approx. 6 per cent) was obtained by using either the base alone (carbowax) or the fungicide (zinc ethylene bisdithiocarbamate)

One must therefore distinguish amongst the ringworm affliction those which are spontaneously curable and those which are not. The latter incapable of responding to local treatment should be submitted to X ray treatment after a period of 1 to 3 months. At the former it remains to find out the extent to which local treatment expedites recovery. In any case it would seem that such a treatment would justify the dissemination of the disease even if it did not effect a cure.

Among the fungicides employed locally are antibiotics and chemicals.

A Antibiotics

In 1946 Cury demonstrated the fungicidal and fungistatic action of tyrothricine *in vitro* upon dermatophytes. In France since July 1944 (at Coudert and Cotte) have attempted to treat ringworm caused by *M. audouinii* with application of tyrothricine (1 mg per g. of ointment) and obtained 41 cures (6 per cent) out of 66 cases in 3 to 4 days. In the remaining cases depilation with X rays was necessary. The correspondence of this with the 6 per cent spontaneous recovery of

Higman and Anderson is noteworthy. In August 1948 however Gate Coudert and Cotte reported further observations extending their work to the trichophyton infections and noting particularly good recovery from those of animal origin. In 1949 Gate Coudert and Jehl confirmed these results and used daily application of a lotion containing 1 mg of tyrothricine per c.c. 70 per cent of acetone and 10 per cent of propylene glycol in alcohol at 70 C. In widespread scalp ringworm however they preferred depilation by X ray.

Moriame (1948) summarized the results of his experiments on the treatment of microsporia ringworm by local application of penicillin and they apparently do not differ from the above.

B. Chemical Remedies

There are so numerous that no attempt is here made to summarize them. In practice they involve the association of a fungicidal or fungistatic substance e.g. phenyl mercurio nitrate salicylanilide eucpram monium hydroxide mixture of a propionate and a caprylate etc. with penetrating base usually carbowax 1800. They give similar results to those obtained with antibiotics. Rens and Warton (1949) have however obtained excellent results with podophyllin but Cridman has blamed this method for causing diffuse baldness (in Higman and Anderson 1951).

Conclusion. There are two types of scalp ringworm those which tend to heal spontaneously and those which do not. The first are treated by the application of a local therapeutic which appears to stimulate natural recovery and prevents the spread of the disease whilst the second are treated by submission to the depilatory action of X ray or possibly of thallium acetate.

In our view the greatest progress has resulted from the introduction of local therapeutic measures in so far as it is now recognized that there may be spontaneous recovery from certain types of ringworm disease which thus do not require the application of X rays.

2. Treatment of Dermatophyte Infections of the Folds and the Glabrous Skin. The dermatophyte infections of the glabrous skin lacking any inflammatory reaction of note may easily be cured by the application of 1 per cent iodized alcohol by Whitfield ointment the salicylic and benzoin acid content being carefully gauged according to the nature of the patient's skin by Castellani's paint or by the application of one of the numerous remedies now available and usually containing unsaturated fatty acid.

The following recipe (Castellani's fuchsin) was proposed by Castellani: pour 10 c.c. of an alcoholic solution saturated with basic fuchsin into 100 c.c. of an aqueous solution of 5 per cent phenol. Filter and add 1 g. of boric acid. Two hours later add 5 c.c. of acetone and after a further two hours 10 g. of resorcinol. Apply once or twice a day. Castellani particularly recommended this remedy for the treatment of tokelau in very primitive natives: it was difficult with civilized patients.

The lesions caused by microd or megaspore have a natural tendency to heal. This is facilitated by the use of one or another fungicide. In a particularly resistant and extensive infection caused by *Ctenomyces asteroides* we had to resort to inoculation by *Trichophyton guineense* a method we believe to have been used by Bruno Bloch. Eight days after this vaccination the patient was cured of a disease which had lasted two months and showed no improvement prior to the inoculation.

A genuine case of Hebra's eczema marginatum usually clears without difficulty by the application of one or other of the fungicides. The forms said to be resistant are mainly erythrasma. However the dermatophytic affections of this region caused by *T. rubrum* are said to defy the rapid measures.

The treatment of dermatophyte infections of the feet or Athlete's foot is the subject of such an abundant literature that we deem it best to indicate our personal experience.

The hygiene of Athlete's foot should be appreciated. Whenever possible the wearing of sandals is recommended (cf. Nicholson, Irving and Melner 1945). The shoes and socks (the latter preferably of cotton) should be changed daily. The shoes may be disinfected by pouring commercial formalin diluted to 1/10 on to a wad of cotton wool, this is placed in the shoe which is enclosed in a box or in wrapping paper for 24 hours. The shoe is then aired before use (cf. Weedman, Emerson, Hopkins and Lewis 1945).

In the chronic form it may be adequate to powder the feet each morning with a proprietary powder or simply with 10 per cent boracic talcum powder. Weedman and Clava (1948) have demonstrated the efficacy of this which has an activity comparable with that of the proprietary remedies.

In the vesicular form a very hot foot bath in water to which an alkaline powder such as Borax Bicarbonate or Thymol 1 per cent has been added is recommended for eight days, night and morning, each immersion lasting 1 minute. The foot bath is followed by careful drying with vigorous rubbing to remove the scale and open the vesicles. At night a proprietary ointment or simply Whitfield's ointment in which the concentration of the salicylic acid varies from 1 to 3 per cent and that of the benzoic acid from 5 to 10 per cent. Apparently it may be advisable to vary the material used on account of the formation of resistant strains (see Villanova and Cavanah 1940). In the morning the pomade is removed by careful washing and a powder is applied for the day.

In the haemorrhagic form rest with limbs extended is not to be indispensable and in any case the patient is in no instance incapable of walking. The same treatment for the vesicular form should be applied but during the day ointment should be used. Foot baths with potassium permanganate (1:4000) are useful in keeping down secondary infection.

It is difficult to be certain whether Athlete's foot is a real and

distinguish relapses from reinfections. We feel that treatment properly carried out for three months will bring about the disappearance of all clinical symptoms and that daily powdering prevent relapses in almost all cases.

3 Treatment of Dermatophyte Infections of the Nails Of all dermatophyte infections onychomycoses are the most difficult to cure. The two main reasons for this are (i) the difficulty of reaching the parasite in the depth of the nail (ii) the well known resistance of *Trichophyton rubrum* which is one of the most frequent causes of ungual mycoses to therapeutic agent.

The treatment of onychomycoses by surgical extraction has long been practised. This method although difficult to get the patient to agree to can be recommended when only one or two nails are affected. Surgical traumatism is thus minimized and post operational treatments have some chance of successful application. Mere removal of the nail is not sufficient to effect a cure. Local application of fungicides is necessary. Afterwards as long as there is any doubt about the healthy growth of the new nail.

Sabouraud in 1930 proposed the abrasion of the outer layers of the nail by means of a dental file. This instrument a scalpel or a nail file must in any case plane away the nail before a fungicide may be applied. One of the most favoured of these is Whitfield's ointment in which the concentration of salicylic acid is increased to 40 per cent. It must be applied carefully ensuring proper protection of the perungual tissues. The treatment must be kept up for six months to one year and the complete collaboration of the patient is necessary for good results.

Nickerson and Whit (1948) proposed the treatment of onychomycoses with ammoniacal silver nitrate which appears to have the property of penetrating the keratin and acting as a fungicide. Out of 16 patients so treated these workers obtained 9 cures and 7 improvements. The number of applications necessary at the rate of one per week varies from 1 to 10. Franks and Sternberg (1950) by the same method reported 7 failures in onychomycoses due to *T. rubrum* and 7 successes in cases due to *C. albicans*. It has already been stated that only the true onychomycoses are caused by dermatophytes and that *Candida albicans* only causes paronychia accompanied by trophic lesions of the nail. In two cases of onychomycoses due to *T. rubrum* we have tried the method of Nickerson and White without success.

Thus the best method of treating onychomycoses seems to be abrasion of the nail followed by the application of a keratolytic and fungicidal substance.

Prognosis

The dermatophyte affections are of a minor character many of them healing spontaneously. Favus which is rare is the most serious because of its duration and the alopecia which it causes. The kerion and the parasitic mycoses are painful but not serious. Lesions of the nails produced

by dermatophytes are unightly and when the nail of the hand are involved they resist most methods of treatment. Athlete's foot which is benign can cause serious loss in man power on account of the number of individuals it attacks.

Differential Diagnosis

A considerable number of cutaneous affections may be confused with those caused by Dermatophytes. Scalp ringworm and tinea are the easiest dermatophytic infections to diagnose after some light experience. However even favus of the scalp may be difficult to diagnose in view of the fact that there are forms of favus without scutula which have been well described by Sabouraud and the number of which is probably increasing. Fichman and Vanbreuseghem (1951) called them asytic favus. Mistakes are made often in connection with the dermatophytic infections of the nails and folds of the skin. In our experience diagnosis of dermatophytic infection is often based upon lesions not of a dermatophytic type and the real dermatophytic diseases are often ignored even by the most advanced observers.

Diagnosis

Five different techniques are used for diagnosing dermatophytic infections each with its own particular value. These involve microscopic examination (of hair scales and nail), culture, experimental inoculation, culture upon isolated hairs and examination under Wood's light.

1. **Microscopic Examination** For microscopic examination to be of value the sampling must first be well carried out; the sampling here is almost more important than the examination itself.

(a) Examination of the Hair

It is easy enough to take with forceps a few hairs broken at the tip of a patch of microspora but this procedure is more difficult with trichophyte infections where the hairs broken off at the level of the papillary orifice are almost impossible to secure. It is better to rub the very itchy patch with the forceps mount the debris in lake taint (with needle) and pick out suspected fragments with the help of a needle. A fragment mounted in chloral lactophenol and examined under critical illumination on the microscope.

In scalp favus diagnosis is easy if there is scutula; one or two are taken with tweezers and crushed in a petri dish. A fragment of the scutula placed in a drop of chloral lactophenol will show numerous striations of irregular shapes. The hair which is the centre of the scutula is examined similarly to investigate the covering filament. In the case of favus without scutula it is usually best to examine the patch with Wood's light and to take specimen of fluorescent hair at the time of the examination.

The new method prepared 90 per cent. positive results for the

examination of hairs is not to be recommended. This alters the disposition of the mycelium in and round the hair & much when a bloral lact phenol has been the filaments intact. Certain mycologists however prefer to use potassium hydroxide for the examination of faecal hair because this is the only



Fig. 24

Mycelium from scale from hair from foot
(100x) (100x) (100x) (100x)
100x 100x 100x 100x

mean of causing air bubbles to appear in the hair and this is thought to be of great value in diagnosis.

Needless to say reproductive structures such as macro or micro conidia are never found in the hair nor in scales and nails. This remark would have been omitted had not recent American work (Appel and Ansel 1949) affirmed that macroconidia (*S. versicolor*) had been found in infected hair. The structures in question were not macroconidia but epithelial cells of the parafollicle which liberated with the hair at the time of its ejection simulate rather poorly the macroconidia of *Sabouraudia*. Work done by Ajello (1951) has already corrected this mistake.

(b) Examination of Scales

Scales are removed with forceps. If they are too small for this the epidermis is scraped with either the blade of an old scalpel or with a

vaccinating stylus over a slide sterilized by heating in a flame. When there are blisters the top of one is torn away or removed with scissors. In contrast with hairs which must be examined in chloral lactophenol scales may be examined in potash after slight warming. Microscopic examination using a high powered eyepiece and a low powered objective enables the moderately practiced eye to scan the preparation rapidly and recognize the possible presence of branching mycelial filament sometimes reduced to arthrospores which provides the diagnosis. There are two possible sources of error—

1 Contamination of the scales by saprophytic *Penicillium* or *Aspergillus* species mycelial filaments of which may be present. These are fairly easy to identify because their diameter often varies considerably in the same preparation moreover in cases of contamination the easily identified fructifications of *Aspergillus* or *Penicillium* are often found.

2 The Mosaic fungus. There is as yet no general agreement as to the exact significance of these curious forms which may be mistaken for the mycelium of dermatophytes. However the shapes of the pseudo mycelia are more geometrical than those of the real mycelium the segments of the false mycelium of mosaic fungus often having sharp or angular sections at their extremities.

Examination of scales in chloral lactophenol is not to be recommended for the turbidity of the preparation obscures the mycelial filament.

(c) Examination of Nails

Nails should be examined in a freshly prepared 10 per cent solution of caustic potash. As with scales chloral lactophenol does not clear them sufficiently. Good sampling is essential with the aid of a scalpel or razor blade and the part of the nail really attacked must be removed. In the symptomatology it has been pointed out that the surface of the nail may remain intact when the nail was internally affected. Thus the external region of the nail must be removed to obtain the diseased part. Bad sampling is undoubtedly responsible for many microscopic examinations appearing to be negative when from the clinical point of view the diagnosis of onychomycosis is evident (cf Dostrovsky, Raubitschek and Saphir 1941). It must be added that this clinical diagnosis is insufficient.

2 Cultures. Cultures should be made on Sabouraud medium with 1 per cent glucose as modified by Langeron. To obtain primary culture more easily it is useful to add penicillin to the medium according to Vanbreughelen technique (1940). As with the microscopic examination it is essential to commence with sample correctly obtained and to try out several inoculations in order to obtain a positive culture. Hairs with a few cultures scales less easily and nails less easily still. Commence with 10 samples of nails which had given a positive result on microscopic examination. Dostrovsky, Raubitschek and Saphir (1941) obtained only 11 culture of dermatophyte. This is an extreme case we

can obtain 50 per cent positive results from the appropriate samples and the percentage would be better still if we could repeat the sampling or had the samples more abundantly available. Westman and Law (1949) who obtained 35 per cent positive results by culturing samples from cases of Athlete's foot insist upon the need to repeat the cultures.

Quite a number of authors emphasize the value of washing scales with alcohol before proceeding with the culturing. Apart from cleaning the lesions with alcohol or ether at the time of sampling, we have also found that there was any need to do this. It is however noteworthy that those who work with tokelau, e.g. Castellani, Ari, Leao and Coto insist upon the procedure.

In our view the one essential is to make a number of cultures. Where it is usually sufficient to inoculate a single tube, at three point order to isolate dermatophytes from hair in the case of scales or nails it is often necessary to deal with six or ten tubes in this way between 1 and 10 inoculations to obtain a positive result.

Three small points in connection with the study of dermatophytes must here be dealt with namely: antagonism between bacteria and dermatophytes; antagonism of dermatophytes between themselves and finally the problem of mixed infection.

(1) Antagonism between Bacteria and Dermatophytes

The question at issue is whether bacteria exert a stimulatory or an inhibitory action on the growth of dermatophytes. Since the experimental results of various workers are so inconclusive a part of the problem clearly eludes us. In general we say however it is clear that when samples for culture are heavily contaminated with bacteria isolation of the dermatophytes becomes difficult. Indirect evidence for this is found in the fact that inoculations upon Sabouraud's medium with penicillin often give more reliable results than those done on media without penicillin.

In 1930 Catalan concluded from numerous experiments that staphylococci favour the development of *T. schoenii*. He wrote however that the development of the fungus is all the greater as that of the microbe is limited. On the other hand Baudet (1932) obtained more abundant and more downy cultures of *S.iform trichophyton* upon an agar medium based upon a staphylococcus culture in ordinary broth which had previously been sterilized by heat.

Against these observations supporting the view that microbial action stimulates the development of dermatophytes are opposed the results of Falck on the one hand and of Vanbreuseghem on the other. Falck (1934) inoculated Sabouraud medium incubated at 37°C. with *pyocyanic bacillus*, a staphylococcus, colibacillus, a *Monilia* and a member of the *Torulopodaceae*. After four days he inoculated into the centre of the culture thus obtained some *T. gypsum*, *steroid*, *T. violaceum*, *V. lanosum* or *A. schoenii* respectively. In all cases there was an inhibitory action, the most resistant fungus being *M. lanosum* and the colibacillus.

being the least active. On the other hand filtrates from cultures of the staphylococcus the colibacillus the proteanic bacillus and the Monilia added to Sabouraud's medium did not prevent the growth of the dermatophytes but rather seemed to favour it. In order to explain the inhibitory mechanism Falch concluded that it involved a direct action of the microbial agents upon the fungi by means of toxins or diastatic action.

In order to simplify the problem Vanbreuseghem (1949) limited it to study of the effect of *Staphylococcus aureus* upon *Trichophyton schoenleinii* and concluded that (i) *Staphylococcus aureus* exert an inhibitory action upon the growth of cultures of *T. schoenleinii* upon conservation media (ii) the inhibitory power varies according to the strains of staphylococcus and of *T. schoenleinii* employed (iii) the inhibitory power is found neither in heat sterilized cultures nor in extract or filtrates (iv) filtrates from staphylococcus cultures do not exercise a favourable influence upon the growth of *T. schoenleinii*.

It is thus evident that the problem of this particular type of antagonism *in vitro* is far from solution. Other somewhat different studies have been carried out with the aim of using the antifungal power of certain bacteria e.g. *Bacillus subtilis* (Lewin and Hopper 1949) and the proteanic bacillus (Balsabanoff 1947).

The antimicrobial activity of the dermatophytes appears in general to be of little consequence. Ciferni (1948) who studied it in relation to staphylococci attributed a maximum activity to *Ichthyophaga violacea* in a medium activity to *F. interdigitale* *F. rubrum* *F. floccosum* and *T. gypsum* a little marked activity to *F. inguinale* and *A. gypsum* a small activity to *T. asteroides* and *A. gallinae* and doubtful activity to *V. audouinii* and *A. schoenleinii*.

(b) Antagonism between Dermatophyte Colonies

This small problem is difficult to summarize because it has so far attracted the attention of research workers so little and the fact so far reported are disparate enough. One may begin with the following fact observed on many occasions and easy to repeat. If a dermatophyte such as *T. crateriforme* or *Sabouraudii* etc. (cf. Vanbreuseghem and Morand 1949) is grown in an agar slant at two points separated by 1 cm toward the middle of the culture medium and half way up the agar slope it will be seen that whereas at first two colonies circular in form will appear as soon as they reach a diameter of 4 to 5 mm the growth will slow down where the edges of the colonies approach one another whilst it increases elsewhere. Between the two colonies there persists a narrow band in which neither colony will grow and it seems that each colony continues to develop in the direction where it finds most free space in front of it.

If instead of only two inoculations one is made every 10 or 20 mm down the whole length of the agar slant a long colony though tending to form a perfect circle will have its growth stopped by the two neighbouring

colonies and only the situated at the ends of the row will be able to develop fully towards the free agar.

These essential facts form a starting point for more extensive observation.

If instead of one dermatophyte different species are used various results may be obtained as has been shown by Dostrovsky and Ranbitchek (1947). These authors consider that *T. gypsum* and *T. asteroides* do not inhibit one another and that *T. schoenleinii* and *T. violaceum* do exert an inhibitory action on one another but not on *T. purpureum* and that *Epidermophyton inguinale* prevents the growth of other dermatophytes (with the exception of *T. purpureum*) as soon as its colony reaches and surrounds them.

Spada (1949) studied the effect of broth culture filtrates of dermatophytes upon the development of other dermatophytes. He concludes that (i) such filtrates are fungistatic towards dermatophytes (ii) the fungistatic power is proportional to the age of the broth culture of the dermatophyte which yielded the filtrate (iii) all these filtrates are not active towards all strains and certain filtrates have a much wider spectrum of activity than others and (iv) the filtrate from one species is active towards several strains of the same species but not towards the one that yielded the filtrate.

It would seem that the fungistatic action of filtrates need not necessarily be related to the antagonism manifested by certain species. This aspect requires much more study before any general conclusion can be drawn.

(c) Mixed Infections

The culture of samples from a diseased region usually results in the isolation of only one dermatophyte species. However, few authors have drawn attention to the isolation from the same lesion of several (usually two) species of dermatophytes: see Catanzaro (1937), Munkathlitt (1941), Franks and Rosenbaum (1940), Loewenthal (1948), Vanbreuseghem and Morame (1948), Mikobertich (1938), Cawley and Horne (1949), Tanaka (1949), Blank (1941, 1941b) and Sabouraud himself (1910).

The number of published instances probably does not reach a hundred and the causes of the effect are quite obscure. Before commenting upon the rarity of these mixed infections information is required as to whether their real frequency which is unknown is less than that of possible infections by each of the isolates: in other words out of 100 individuals with favus how many would have been infected by the *T. violaceum* found on one of them in association with *T. schoenleinii* if none had had the favus infection. In the absence of data no answer to this question is forthcoming.)

3. Experimental Inoculation. Inoculation of dermatophytes into laboratory animals is rarely used for the identification of the fungus but usually to study immunity and the nature of the pila lesion.

The guinea pig is the preferred animal and has been the most useful. The disease develops in it 1 day after an incubation period of not 10 days; there is little to see for a month after the inoculation. All dermatophyte species do not give the same results. Some such as the



Fig. 1
Guinea pig inoculated with *T. mentenii* 10 days after inoculation. Lesion 10 days after inoculation.

C. moniliformis are always inoculated successfully without exception, even consistent result or failure. Inoculation experiments with *T. mentenii* are rarely profitable.

Inoculation techniques vary little. All aim at an intimate contact between the skin of the guinea pig and the dermatophyte for the inoculation to have some chance of success. Sabouraud and his associates introduced a parasitized hair into a small wound in the skin of the guinea pig, or by rubbing a ruled culture over the skin. Talbot (1911) and Cabaza (1911) amplified the particularly difficult inoculation of *T. ferrugineum* into the guinea pig by means of the scales produced by erythrocutaneous. Our own preference is for Rye's technique (1911).

which is simple and which we found effective on the dead. According to I. H. C. A pulverized portion of a fully developed (3 week) culture on 5 bouvard agar is removed and mixed in mortar with small quantity of honey. The virulent paste is applied to a healthy guinea pig over a piece of skin with the dimensions of a five franc piece and simply cut with the scissors. The rod is the only instrument necessary for this application. The resultant infection is constant and I have had no failure amongst 4 guinea pigs inoculated. It must be noted however that this consistency of Ivalier's result was due to the fact that he worked with *Ctenomyces griseus* and all other species do not work so well.

The age of the culture used for inoculation seems to be of some importance though no general data are available on this. After failure in our attempt to inoculate guinea pigs with young cultures of *Laetisaria ardens* (Vanbreuseghem 1949) we recalled Caturen's success in inoculating the dermatophyte into guinea pigs and Algerian monkey by using old cultures. Repetition of our experiments with old cultures transformed failure into success.

The effect of inoculation of the guinea pig with a dermatophyte are apparent from the seventh to the tenth day when erythema and desquamation are evident. Examination of the scales reveals mycelial filament and little later the hairs are invaded. These fall eventually. Towards the third or fourth week the only sign of the inoculation is a glabrous plaque over which the hair soon grows again.

4 Culture of Dermatophytes in vitro on Isolated Hairs. According to Vanbreuseghem (1949) this culture method permits the fact to be established independently of morphological characters that the rod is indeed a dermatophyte. From the 10 days after the introduction of a suspected growth upon hair the destruction of the hair by the fungus becomes apparent either by destruction of the cortex toward the centre or by the formation of perforation organs which enter the hair and divide into many segments. Vanbreuseghem in applying this technique to the study of numerous dermatophyte species has shown that most of them can produce such lesions and that they stand alone amongst the fungi in this respect. This is a new diagnostic method available and a useful technique for studying the microscopic morphology of the fungi.

5 Wood's Light. This type of light results when ultra violet rays of length 3650 Å approximately pass through glass filter—Wood filter—containing nickel oxide. In the dark this radiation causes various substances to fluoresce. Margat and Devise discovered that hair infected by ringworm fluoresces in Wood light. This is consequently used in diagnosis in following the treatment of patient and in the detection of sources of contamination.

When a hunk of hair affected by microspora is examined in Wood light the patches of *Trichosporon* exhibit a greenish fluorescence. Infected hairs isolated amongst healthy ones or which have fallen on the clothes are easily recognized.

of the urticaria type or a retarded reaction of a tuberculin type. The reaction appears immediately after the injection. It is less frequent than the retarded reaction which commences after 24 hours and takes the form of a papule surrounded by an erythematous zone sometimes reaching 10 cm in diameter. The reaction reaches its maximum toward the second or third day and usually disappears toward the fourth or fifth day sometimes later leaving a darkish pigmentation and a slight desquamation. This local reaction may be accompanied by a general one—a moderate temperature discomfort polynuclear neutrophilia and relative leucopenia and a regional reaction (inflammatory conditions at the site of the mycosis). In non-sensitive individual the injection of trichophytin produces a lymphocytosis (cf. Casalsky).

All dermatophytes do not sensitize equally. Individuals with favus for example even when the attack is very serious show no reaction. Strong sensitization accompanies dermatophytic infection of animal origin. *Trichophyton rubrum* very little whilst *Epidermophyton floccosum* is known to be one of the most active sensitizers.

The trichophytin reaction has only one group specificity, i.e. it can be induced by trichophytin prepared from any dermatophyte in a person affected by a very different dermatophyte. Its diagnostic value is thus very limited. It is positive in a certain number of individuals apparently free from dermatophyte infections. Mukatblitt and Director (1933) found it to be positive in 181 (60.3 per cent) out of 300 clinically attacked by a dermatophyte infection and in only 24 (4 per cent) out of 590 apparently unaffected individuals. It is generally admitted that there is a higher percentage of positive reaction in the control experiment the result of American workers are in any case corrected by the fact that the percentage of positive reactors reaches 7.3 per cent in cases where clinical diagnosis was confirmed by microscopic examination or culture and falls to 4 per cent in those cases where the examination was negative.

A positive reaction may be found ten years after the infection which caused it.

Sensitivity to trichophytin can be passively transmitted by injecting serum from a positive reactor into the skin of a negative reactor (Prausnitz-Kustner phenomenon). The reaction would not matter in using the serum of an individual giving an immediate reaction of the urticaria type. According to Lilson and Huppert (1919) the retarded reaction—tuberculin type—is the first to appear marked by the tubercular antibodies. The precocious reaction—urticarian type—appears later and is rendered obvious by the circulating antibodies.

Once a source of dermatophyte infection is installed in a part of an organism it can move for a certain distance but besides the lesion produced by the immediate extension of the parasite certain others which Bruno Bloch gives the name trichophytoid appear at a distance. Usually appearing on the tenth day of the infection (what is trichophytoid?)

correspond to a state of sensitivity induced by trichophytin in fact it is agreed that they appear only in positive reactors

Trichophytids arise abruptly and exhibit a symmetrical distribution. They have a variable appearance knotted erythema scarlatiniform exanthema polymorphic erythema erythematopustular eruption dyshidrotic lesions. Injection of trichophytin in a person with trichophytid produces at the position of the injection trichophytids with the same clinical aspect as the pre-existing ones

The exact cause of trichophytids is still unknown. It is thought that they may arise through the passage of the fungus through the blood or of sensitising substances produced in the centre of dermatophyt infection. The symmetrical localization of the trichophytids suggest that they originate from the blood. The fact that in certain cases it has proved possible to isolate the fungus from the bloodstream of patients with trichophytid favours the parasitic nature of their formation. On the other hand the evidence of a toxin in the blood has never been demonstrated (Peck 1940). Certain writers hold that trichophytids may arise by the contact of healthy but sensitized skin with the seat of the infection but the characteristic symmetry of the lesions opposes this view.

Trichophytids are usually sterile and nearly all attempts to culture from them have failed. Moreover it must be admitted that the fact that lesions yield cultures is proof that they cannot be classed as trichophytids.

The trichophytid concept has rapidly become very popular. From there it has been easy to pass to that of idiosyncrasies in general and the microbe in particular to designate all the forms of an allergic nature appearing at a distance from a seat of infection responsible for them. According to Peck (1940) the following are the criteria which permit a diagnosis of microbe —

(i) The organism is possible must be demonstrated in what is considered to be a classical manifestation of the disease e.g. lesions on Athlete's foot

(ii) The organism isolated must be pathogenic. This however is not absolutely essential

(iii) A reaction similar to that of tuberculin or trichophytin must be detectable

(iv) What is regarded as a microbe must frequently accompany the primary lesion

(v) The same organism as is isolated from the primary lesion must be obtainable by haemoculture. (It is feared that this criterion is however only a laboratory criterion or verifiable)

(i) The microbes must appear after the primary lesion

(ii) The microbes are usually sterile

(iii) The diagnosis of microbe is supported by —

(i) Their appearance in the form of multiple disseminated elements

(b) a tendency toward the symmetrical disposition of the element

(c) a tendency towards spontaneous recovery after healing of the primary centre of infection

From this idea of cutaneous trichophytid there is a tendency to pass to that of vascular lesion. Even though the nature of the former seems to be readily acceptable much has still to be done before there can be any conviction about the latter. Peck (1930) divided the trichophytid into four groups—

I Epidermic trichophytid (attacking the epidermis)

- (a) Eczematoid (dyshidrotic)
- (b) Ichthyoid
- (c) Parakeratous
- (d) Psoriasisform

II Cutaneous trichophytid (particularly attacking the papillary body)

1 Diffuse forms—

- (a) Scarlatiniform exanthemas and enanthema
- (b) Erythrodermia

B Circumscribed and disseminate forms—

- (a) Follicular localizations usually healed
- (b) Localizations not exclusively follicular—maculopapular and venous exudative eruptions
- (c) Erythematoid forms

III Subcutaneous trichophytid (hypodermic nodules of knotted erythema type)

- (a) Acute form tending to heal
- (b) Chronic form tending to increase

IV Vascular trichophytids

- (a) Venous phlebitis migrans
- (b) Capillary urticaria
- (c) Purpura

The idea of vascular trichophytid seems to lack any firm support. Peck however goes so far as to affirm his mycosen phlebitiform ones which he attributed to vascular trichophytid. Claim of a relation upon the causal relationships between the dermatophyte infection and Thrombo Angitis Obliterans have either been forgotten or reported with very little confidence. They may usefully be recalled because of their theoretical interest and because they merit verification. Thompson (1911) was the first to attempt to establish a relationship between Athlete's foot and Thrombo Angitis Obliterans (T.O.) At almost the same time (1911)

Meyer Naede published his observations of 30 patients with T O *8 (66 per cent) were suffering from Athlete's foot and in 20 of these the dermatophyte infection was imported. Observation of 30 control cases revealed the existence of dermatophyte infections in 22 (73 per cent) of which 9 were of serious nature. Cutaneous reaction with trichophytin was positive in 14 patients suffering from T O (80 per cent) and notably in 1 patient clinically free from Athlete's foot whereas only 6 of the controls (20 per cent) reacted to trichophytin. Ten patients were observed during acute attack of T O the attack was preceded by aggravation of the Athlete's foot in 7 of them. Meyer Naede vainly attempted to explain the predominance of T O in males though females are equally susceptible to Athlete's foot on the grounds that this disease is usually less serious in women and is less frequently accompanied by a positive trichophytin reaction. Reiss and Graham (1946) using technique devised by Reiss (1944) were unable to detect scarification in estrated rabbits infected by *T. purpurum* for 1½ years. More recently Holman (1947) claimed to have obtained interesting result by treating two cases of T O with trichophytin injections.

So far as we know the problem rests at this point. It would not have been presented had not certain clinical facts recently observed by us given them a possible clue.

9 *Therapeutic Utilization of Trichophytin* Cutaneous sensitivity towards the injection of trichophytin only develops if dermatophytes parasitize the skin (Bulbinger 1940) it does not appear even if the dermatophyte is found in deeply seated organs. Rivalier (1929) observed in the guinea pig, the formation of a chronic subcutaneous abscess after inoculation by a living *Trichophyton* without any kind of resultant immunity or allergy. Rivalier has on the other hand attempted unsuccessfully to desensitize guinea pigs by means of intradermal injections of trichophytin. This worker (1936) thus considers the therapeutic action of trichophytin to be illusory.

These facts and other analogous ones have discredited trichophytin the possession of antigenic properties and have caused the substance to be regarded only as a reaction.

It is however to be noted that repeated injections of trichophytin terminated without accompanying cutaneous reactions even if at the time of the first injections they induced considerable reactions. The conclusion would not hold good and it would appear to be impossible to sensitize man or animals to trichophytin by repeated injection of the substance. Rivalier (1929) wrote "It may well be that trichophytin—analogue in this respect to tuberculin—behaves as a poor antigen capable of being used for the detection of a pre-existing state of sensitization but incapable of inducing similar state. However like tuberculin trichophytin is able to bring about desensitization and as such deserves to rank in the therapeutic arsenal against dermatophyte

CHAPTER IX

Haplomycosis

EMMONS (1948) gave the name haplomycosis to a fungal disease of rodent caused by *Haplosporangium parvum* Emmons and Ashburn 194. This organism was isolated from the lungs of 64 per cent of the pocket mice (*Perognathus*) examined by Emmons and Ashburn (1948) whilst investigating the incidence of *Coccidioides immitis* amongst the rodent in the region of San Carlos Arizona. Other rodents were found to be naturally infected (e.g. *Dipodomys*, *Citellus* etc). In Canada Dowling (194) found 14 infected animal out of 271 rodent taken in Alberta. Characteristic spherules were encountered in the lungs. Thirteen of the infected animal were *Peromyscus* (*Peromyscus maniculatus borealis* white footed deer mice) and one was a red squirrel (*Sciurus hudsonicus baileyi*). Of these 14 cases, *Haplosporangium parvum* was isolated from 5. Whereas in Arizona the greatest dimension of fungal cell obtained was 45μ in Alberta spherules as large as 200μ with wall thickness 8 to 10μ were seen.

The lesions in rodent haplomycosis are discrete and confined to the lung tissues. They usually form a poorly developed granuloma. Analogous lesions were experimentally reproduced by intranasal inoculation into white mice by Ashburn and Emmons (1945). The experimental lesion however exhibited a tendency towards spontaneous regression after 4 to 6 months. Ashburn and Emmons nevertheless consider them to be more than a mere tissue reaction in response to the presence of foreign bodies. They support their view as follows: (i) by the fact that killed spores do not produce this reaction by the appearance of a perivascular lymphocytic infiltration at a distance from the granulomatous focus; (ii) by the slow but regular growth of the lesions for 4 or 6 months.

Within the tissues of naturally infected rodent the pathogen appears as rounded cell which attain a diameter of 10 to 14μ . As noted by these dimension may be very much greater. Fungal hyphae are characteristic of *Coccidioides immitis* but have not been observed. The protoplasm dense non vacuolated and basophilic. In the lungs of experimentally infected mice the fungal structure attain a diameter of 40μ after two months. These spherules are enveloped by a thick membrane having several layers, this includes a large eosinophilic nucleus 5 to 10μ wide containing a basophilic nucleolus. There is no endospore formation. In short the spherules resemble young spherules of *Coccidioides immitis*.

On an acid dextro agar *H. parvum* grows more slowly than *C. immitis* and at first appears as a glibrous and colourless disc. The centre and then the periphery rather rapidly becomes downy. The down which is at first white browns with age. Numerous individual variations may be found from strain to strain certain colonies remaining wholly or partly glibrous. The but slightly segmented mycelium is rather well branched. The branches arise at random from the main hypha without any reference to septation. The hyphae are at least 1μ in diameter and occasionally may attain 4μ .

The spores are somewhat rounded 3 to 3.5μ in diameter with an apiculate membrane though like spores may have a complete smooth wall. They are borne singly or in short chains of two or three at the end of hyphae or upon more or less branched lateral sporophores. Emmerson and Ashburn (1941) regard these spores as monosporous sporangia or sporangia which they call conidia. These conidia are attached to hyphae or conidiophores by a delicate filament from which they are separated at maturity by a septum.

Dowding has obtained laboratory cultures on soil agar from Canadian strains and has evidence that the sporangia are not readily detachable from their sporophores but are very adherent. She considers it probable that when they die they burrow.

H. plasmoparvum parvum belongs to the genus *Haplosporangium* Thaxter 1914 containing three known species namely *H. b. parvum* Thaxter *H. decipiens* Thaxter both isolated from horse dung and *H. lignicola* Martin 1937 isolated from rotten wood. *H. parvum* is differentiated from these three species by its much smaller spores.

Dowding (1947) believes that *Blasomys dermatitidis* and *Histoplasma capsulatum* may have an affinity with *Haplosporangium parvum* (occasional *immitis* might have had less obvious connection with these except for the fact that Emmerson considers it to be a *H. b. parvum* with monosporous sporangia though he has obtained from cultures sporangia enclosing two or three spores.

Emmerson and Ashburn (1941) prepared a substance called haplosporidin which produced a positive reaction in 20 out of 33 mink which were reacted positively to encysted *Toxoplasma*.

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CHAPTER X

The Histoplasmoses

It is possible to distinguish human histoplasmosis or Darling disease, epidemic lymphangitis of the Equidae and perhaps histoplasmosis of rodents. The first of these will be dealt with more fully than the other two which will be only outlined.

A HISTOPLASMOSES IN MAN

Definition

Histoplasmosis is a mycosis of the reticulo endothelial system caused by *Histoplasma capsulatum* Darling, 1906. It is characterized by anaemia, leucopenia and hepatosplenomegaly. The denomination of this mycosis has rarely been in question it is sometimes called Darling disease.

Historical

Histoplasmosis was first recognized by Darling, in 1906 whilst seeking cases of kala-azar in the region of the Iquitos estuary. The organism found in the tissues was regarded as a protozoan and named *Histoplasma capsulatum* by Darling in 1906 when he discovered two new cases. In 1919 da Rocha Lima in a comparative study of sections from histoplasmosis lymphangitis of solipeds and kala-azar came to the conclusion that the agent of histoplasmosis was not a protozoan but a fungus. The fourth case of histoplasmosis is the first to be found after Darling's report was described by Riky and Watson in 1920. It was not until 1934 however that Monbrun confirmed da Rocha Lima's findings by cultivating the pathogen from the blood of a young child whom Dodd and Tomkin (1934) had recognized during his lifetime to be subject to histoplasmosis. The mycological study of the organism carried out by de Monbrun merely left to his successors the problem of its systematic. De Monbrun contributed further essential facts by isolating the pathogen from a dog and demonstrating that it may be inoculated into young puppies. This author further proposed that the name Darling histoplasmosis should be replaced by Darling's cytomycosis. Further contributions to our knowledge of histoplasmosis were made by Van Peltus, Demmon and Hollinger (1941) and also Zarafonitis and Landberg (1941) who demonstrated the cutaneous sensitivity to histoplasmin of patients suffering from histoplasmosis. These authors regarded this reaction as specific. The important work of Fimmons and his school on the etiology of the disease will be given later.

Importance and Geographical Distribution

Like the other great visceral mycoses histoplasmosis is rare, the literature recording about 100 cases. It attacks without racial distinction individuals of two age groups—early infancy and the fourth, fifth and sixth decades. Whereas children are affected regardless of sex histoplasmosis in adults affects twice as many men as women. There is a slight preponderance of cases amongst agricultural workers. The youngest case on record is undoubtedly that of Schlumberger and Service (1944) who diagnosed histoplasmosis *pre mortem* (by aternal puncture) and *post mortem* in a male child seven weeks old on entering hospital who died two weeks later from hepatosplenomegaly and anaemia.

The histoplasmosis cases are mainly found in the states of the Middle West of the U.S.A. (Missouri, Minnesota, Michigan) but it is a disease of world-wide distribution. Cases are on record from the following regions: South Africa, South America (Argentina, Colombia, Brazil, Uruguay), England, Austria, Hawaii, Java, Mexico, the Philippines, the Sudan, Venezuela.

Etiology

The etiology of histoplasmosis is not yet known with certainty, but work in progress suggests that it is a disease of rodent which may be transmitted to dogs, cats and man. De Monbrun (1939) first discovered a case of histoplasmosis in the dog; he showed the strain of *Histoplasma capsulatum* isolated to be identical in all respects with the isolated from man and that it could be inoculated by mouth to young dogs. In 1941 Emmon, Bell and Olson published an account of their work in Virginia. They captured 160 rodents in the Loudoun County region where four cases of human and three of canine histoplasmosis had been recorded and demonstrated the presence of *Histoplasma capsulatum* in one mouse and ten rats. Large anatomico-pathological lesions were not found in the animals from which the fungus was isolated and the parasite was mainly isolated from the liver and the spleen.

The method used by Emmons and his co-workers in making these isolations consisted of performing an autopsy with all related instrument upon newly killed animal, the abdomen having first been covered with a solution of cresol. Fragment of viscera (pleen, liver, suprarenal glands, bladder, kidney) were then cultured on Sabouraud's medium whilst other fragments of the same viscera were preserved in formalin until after to 4 weeks incubation at 30°C the result of the culture was positive or negative.

Olson, Bell and Emmons showed at about the same time (1947) that they could obtain cultures of *H. topasium capsulatum* from eroded tick (*Dermacentor variabilis*) previously fed on a dog naturally infected with histoplasmosis. However, four dogs placed in contact with the first one showed no disease symptoms.

In 1949 Emmons doubtless inspired by his success in his study of

coccidioidomycosis isolated two strains of *H. capsulatum* from 387 specimens of earth taken from a farm where he had captured 7 rats infected with histoplasmosis. Further he demonstrated the presence of macroconidia or echinulate chlamydospores characteristic of *Histoplasma capsulatum* in aqueous suspensions of the two specimens which yielded these strains. Thus it is evident that this parasite find in soil a medium favourable to its reproduction in the saprophytic state.

Pathogenic Agent

The binomial *Histoplasma capsulatum* was proposed by Darling, in 1906 when the organism was known only in its parasitic form and was moreover regarded as a protozoan. In spite of this confusion at the beginning there is no good reason for not retaining Darling's name for the agent of histoplasmosis. De Monbreun has took this view when as the first to isolate the parasite he furnished a complete description of it.

However the following synonyms are encountered in the literature—

<i>Cryptococcus capsulatus</i> (Castellani)	<i>Funaria capsulatus</i> (Darling)
and Chalmers 1933	Moore 1934
<i>Traubmannia capsulata</i> Almes	<i>Sporobolus</i> sp. H. Mann and
1933	Schenck 1934
<i>Funaria parviformis</i> Monte	<i>Histoplasma parviformis</i> (Monte)
1934	Dodg. 1935

Stained by Wright or Giemsa *H. capsulatum* appears in the blood marrow or the spleen as a round or oval body with a greatest width of 1 to 4 μ . In most cases it is an intracellular inclusion within the cells of the reticulo-endothelial system in macrophages and giant cells. It is enveloped by a refringent capsule of variable thickness which does not stain by the usual procedures. One end of the cell is often less thick than the other, buckling occurs at this acuminate extremity. It is the occurrence in the widest region of a crescent shaped mass of chromatin that supports a resemblance between *H. capsulatum* and a *Leishmania*. However the absence of blepharoplast distinguishes it from a Leishman Donovan body.

The isolation by Monbreun of the parasite in pure culture from the blood of chick infected with histoplasmosis demonstrated the dimorphism of the organism which has a yeast like aspect in the parasitic condition but is filamentous in culture. It is in fact possible to obtain both phases in culture. The yeast like phase called YP by American workers has the same morphology as that of the organism in the tissue whilst the mycelial phase (MP) has the aspect of filamentous fungus.

There is no outstandingly characteristic feature to distinguish colonies of *Histoplasma capsulatum*. The yeast like phase appears as small brilliant whitish colonies whilst the mycelial phase appears as whitish down which assumes a brownish colour after two weeks.

The colonies of the mycelial phase are made up of segmented branched

De Monbreun drew attention to the need for using hydrochloric and not acetic acid to adjust the pH of the culture medium. In fact acetic acid as well as small amount of sodium acetate prevent the development of the fungus.

Colonies of the yeast-like phase are made up of round cells 3 to 4.5 μ long which bud only at their acuminat end the buds remaining attached for some time to the mother cell by a thin filament often as much as μ long. In stained preparations the chromatin is in the form of a crescent near the rounded end of the cell. There is thus no essential difference in the appearance of *H. capsulatum* in the tissue and in culture of the yeast-like phase.

It is just as difficult to get and keep the yeast-like phase as it is easy to lose in the mycelial phase. There appear to be four necessary conditions: (1) a temperature of at least 37°C. for below that temperature the cultures revert inevitably to the mycelial phase; (2) a medium rich in protein, preferably containing rabbit blood or that of another animal, human blood being excellent; (3) a high degree of humidity, drying, causing reversion to the mycelial phase; and (4) a CO_2 for the nature of which is not yet fully determined, but which probably involves the level of CO_2 concentration in the atmosphere surrounding the culture. It has hitherto been correctly thought necessary to seal the culture tubes hermetically to obtain the yeast-like phase of *H. capsulatum*. This doubtless not only ensures the maintenance of a certain degree of humidity, but also establishes a sufficiently high concentration of CO_2 in the cultures, a factor of even greater importance. Indeed, according to B. Ben, the yeast-like phase of *H. capsulatum* as well as that of *H. farciminosum* may be obtained by culturing in an atmosphere containing 1 to 20 per cent CO_2 . The CO_2 may not be replaced by nitrogen and a lowering of the percentage of CO_2 to below 15 per cent causes reversion to the mycelial phase.

In order to obtain the yeast-like phase other less obvious conditions are necessary. In particular it is essential to culture on solid media. Salmon (1947) was unable to obtain the yeast-like phase on liquid media such as broth, serum or broth containing 1 per cent serum. He obtained it however on a semi-liquid medium with the following composition—

Proteose peptone Dif	10 g
Neo peptone Dif	3.2 g
Tryptone	3.2 g
Glucose	8 g
Sodium chloride	5 g
Dipotassium hydrogen phosphate	0.1 g
Agar	1.5 g
Distilled water q	1,000 ml

On this medium the optimum pH for growth is between 6.1 and 8.1. The temperature of 37°C. is essential for at 2 or 31 there is a reversion to the mycelial phase. Optimum viscosity is obtained with 1.75 g. of agar.

in 1000 ml but the agar may be replaced by a silicon salt. According to Salvin perfect development is obtained under anaerobic conditions in mixtures of 10% 40 and 50 per cent of CO_2 with air and least in 100 per cent oxygen. Salvin claims that this medium is better for retaining the yeast like phase than one containing blood.

Further all workers agree that the yeast like phase can only be maintained by frequent transfer every 3 to 4 day.

As has been seen the addition of blood to the medium is not absolutely necessary. The yeast like phase can be obtained on a serum medium or on Pitragnani's medium without malachite green.

The transition from the yeast like to the mycelial phase has been observed by de Monbreun by transferring a culture of the yeast like phase to an agar plate (Sibouraud's medium) at laboratory temperature. The yeast like forms enlarge and round up until they reach 4 to 5 times their original diameter. These cells give rise to a mycelial filament which separates and yield secondary filaments.

A number of workers have been interested in obtaining the yeast like phase from the mycelial phase with rather contradictory results. Only Ciferri and Redaelli (1934) and Conant (1941) appear to have succeeded in culturing *H. capsulatum* at 37°C on a blood medium in sealed tubes. According to Campbell (1947) it is quite easy to recover the yeast like phase by transferring every 2 to 3 days from the mycelial form kept at 37°C to the following medium which is a modification of Francis's medium (cf. sporotrichosis)—

Veal broth	1 (100) ml
Rabbit or horse blood	50 ml
Peptone	10 g
Glucose	10 g
Sodium chloride	5 g
Cysteine or cystine hydrochloride	2 g
Agar	50 g

The agar salt and peptone are added to the broth which is heated until the agar dissolves. The cystine is dissolved in sufficient water and solution to adjust the pH to 6 to 8 then added to the mixture. This is sterilized at 120°C for twenty minutes and after cooling to 40°C the blood and glucose are added under sterile conditions. Shake repeatedly for 3 hours at 60°C and tube out.

It is certain however that when only the mycelial phase is available the easiest way to recover the yeast like phase is to inoculate into an experimental animal and to re-examine isolation of the yeast like phase from the diseased organ.

Symptomatology

It is difficult to give a precise picture of all the symptoms of histoplasmosis but in some it is worth recalling its similarity to kala-azar.

g anaemia leucopenia splenomegaly emaciation and prolonged fever. The disease persists from some three weeks to 8 months and in exceptional cases for 4 8 10 and even 16 years.

In a child the most important signs are temperature digestive troubles and diarrhoea followed by obvious hypertrophy of the spleen and liver and also of the lymph nodes. Death follows a state of advanced cachexia.

In adults the disease often takes a slower course and extensive involvement of certain organs may give a strong bias and render diagnosis difficult. According to Miller *et al* (1947) 60 per cent of the cases show lesions of the skin and of the mucous membranes. According to these workers the extraneous mucous manifestations may be grouped in 5 categories—

1 Ulceration and granulomatous tumours of the buccal mucosa (29 of 4 patients exhibiting cutaneous lesions).

2 Papular patches cutaneous ulceration (11 out of 4 patients).

3 Purpuric lesions capable of ulceration (1 patient).

4 Abscesses furunculoid lesions (rare).

5 Localized or generalized dermatitis (rare).

Pulmonary manifestations are frequently noted as well as gastro-intestinal trouble such as diarrhoea vomiting haemorrhage perforation. According to Parsons and Zarafonetti (1941) lesions of the middle ear were found in 8 cases and in one of Crumrine's cases the parasite was found in the pus from an otitis media.

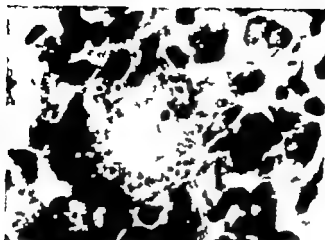
Other diseases able to reach the reticulo-endothelial system may be found associated with histoplasmosis. This is particularly true for tuberculosis (5 cases) and Hodgkin disease (1 case). Confusion with the latter is possible even from the histological standpoint and several authors (Miller *et al*) insist upon the presence of cells strongly reminiscent of Sternberg-Reed cells in tissues attacked by histoplasmosis.

Two good fundamental works on the symptomatology of histoplasmosis are those of Parsons and Zarafonetti (1941) and of Lark (1946).

Histopathology

Histoplasma capsulatum is essentially an intracellular parasite found mainly within the cells of the reticulo-endothelial system. Its presence conditions the formation of granulomatous processes ending with necrosis ulceration or calcification. The parasite is found most readily in the liver spleen lung lymph nodes and bone marrow. Duart (1941) demonstrated it in the central nervous system and the meninges. He considers that if the parasite has not been more often described in the encephalon and its annexes it is because it has not been systematically looked for.

Destruction of hepatic cells with centrolobular necrosis has been observed in the liver. The sinuses are filled with tightly packed macrophages as well as Kupffer cells of the parasites.



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Hyalopis *p. n. t.* (H. p. n. t. H. p. n. t. H. p. n. t.)
The of H. *p. n. t.* (H. p. n. t. H. p. n. t. H. p. n. t.)
p. n. t. *p. n. t.* (H. p. n. t. H. p. n. t. H. p. n. t.)



14

```

// try to get the last element
if (vec.back() != 0)
    return 0;
}

```

In the lung there is massive infiltration by large monocytes and the alveoli are collapsed.

It will be noted in general that the tissues are invaded by large mononuclear element and that there is no polymorph infiltration.

Treatment

There is no effective therapy in histoplasmosis. However a few cases of recovery have been reported when sulphadiazine was used. It is interesting to note that Campbell and Shaw (1951) demonstrated the ineffectiveness of streptomycin injection in mice infected experimentally with *H. capsulatus* and this antibiotic has a stimulating action upon the mycelial phase *in vitro*. On the other hand the same authors showed that atabrine afforded partial protection to mice against *H. capsulatus*.

Prognosis

The fatal prognosis in children must not be applied without reserve to adults. It seems that in certain cases the disease may become limited after a process of fibrosis. Some cases of recovery have been claimed but in practice these are better disregarded.

Differential Diagnosis

Tuberculosis, leukaemia, kala-azar and Hodgkin's disease should be differentiated from histoplasmosis. There is a report of a wrong diagnosis of amoebiasis in a case in which intestinal troubles were predominant. There may be possible confusion with Hodgkin's disease on account of the presence of element resembling Sternberg-Reid cells in the tissue. Park (1948) reported the formation in the liver of accumulations of parasitised strongly similar to the pseudo-cysts produced by toxoplasma. Quite clearly no clinical argument is likely to replace the demonstration of the parasite in smear preparations or sections or its isolation through culture.

Laboratory Examination

The blood exhibits moderate anaemia of the hypochromic type with red blood count of 3 to 4 million per cmm. Leucopenia is frequent but not general and sometimes associated with a relative lymphocytosis.

The blood proteins show an alteration in the albumin globulin ratio with relative increase of the latter.

Albuminuria is frequent. The urine may yield red and white cells and the organism has been cultured from urine.

Mycological Diagnosis

Mycological diagnosis in all cases searching for the pathogen and isolating it in culture.

1. Search for the organism in the blood is rarely successful for the histoplasmas are confined to the monocytes. It is more profitable to search

marrow obtained by sternal puncture. The same also applies to smear preparations made from sections of portions removed by biopsy. Staining of blood or marrow smears is carried out with Wright or Giemsa.

The demonstration of *H. capsulatum* in sections is effected by the usual basic stains namely: cohn haematoxylin, Mason's trichrome, Van Gieson, Goodpasture, Gram Weigert and Giemsa. The capsule may be stained by Heidenhain and most silver impregnation methods.

For distinguishing histoplasma from leishmania Park recommends the use of Gram's method as modified by Krajan (1917) or the Fuchs method (1931). With Krajan the histoplasmas are violet or greenish blue whereas the leishmanias are pink red. With Fuchs the histoplasmas are stained blue and the leishmanias mauve. It is noteworthy that the parasite form is Gram positive and slightly acid fast.

Isolation of the parasite by culture is undoubtedly the best method for diagnosing histoplasmosis. Parsons and Zarasenetis (1911) indicated that in 23 instances where the pathogen was cultured positive cultures were in 18 obtained from tissues, in 9 from blood and in one from marrow. It appears that whereas search for the pathogen in blood smears is usually negative it can be isolated often enough by blood culture.

Inoculation is carried out on Sabouraud's medium at 37°C or at 25°C on blood medium in sealed tubes. The culture tubes must be retained for four weeks on account of the slow development of the parasite.

In a case of generalized histoplasmosis Dublin (cultivation) and Friedman (1919) were twice able to culture the pathogen by removing 5 ml of blood in a syringe containing 1 ml of sodium citrate. The mixture was kept at laboratory temperature and two weeks after removal of the blood mycelium appeared above the layer of white globules.

Experimental Inoculation

It is too frequently ignored that Disinger successfully attested animal inoculation of histoplasmosis. Having failed to culture the organism he was limited to using infected human tissue as his source of material for inoculating guinea pigs. De Monbreun was more fortunate having all his disposal cultures of *H. capsulatum capsulatum*. From the yeast like phase and by means of intravenous injection he produced in two monkeys (Morris and a) lesions similar to those found in man and also marfanoid anaemia, lymph node hypertrophy and hepato-splenomegaly. Death occurred 14 days after inoculation and the pathogen was recovered from the monocyte of the circulating blood during the life time of the animal but from the tissues after death. Failure attested the use of the mycelial phase to inoculate a monkey.

Inoculation of an animal with a culture from the mycelial phase is not like phase permit the recovery of the pathogen from various organs after several months. Except in certain cases however the lesion did not become generalized in man and in most cases the animal recovered. According to Freeman, Bell and Olson (1941) mice and guinea pigs do

not exhibit any further symptoms after the acute phase of the infection but it is possible two years after the inoculation to isolate the pathogen from the liver and spleen. The dog which is one of the animals spontaneously infected can easily be infected in the laboratory even through the digestiv tract (De Monbreun). However the lesions do not become generalized. Brandt (1940) showed that it is possible to induce a generalized effect if the inoculation is preceded by X irradiation of the animal.

Catani (1941) working with an African strain observed no generalization in the mouse and the guinea pig but he was able to isolate the pathogen from the spleen two months after inoculation.

Allen (1948) inoculated histoplasmosis into mice and guinea pigs via the nostril and the ear and the result was positive in 70 per cent of the mice and 75 per cent of the guinea pigs respectively. He concluded that in nature infection occurs via these two routes.

The varying results obtained by different workers tend to support the view that there are considerable differences in the virulence of the different strains of *H. capsulatum*.

Histoplasmin

First prepared in 1943 by Van Pern, Benson and H. Singer histoplasmin is a metabolic product of *H. capsulatum* developed in the culture medium. Its intradermal injections produce reactions similar to those of tuberculin. It soon became apparent that histoplasmin inoculation not only gives a positive reaction in those attacked by histoplasmosis but also in apparently healthy individuals. To some extent this has emerged from the very numerous epidemiological studies that have attempted to clarify the real significance of this reaction—

1 The extent to which the histoplasmin reaction is positive varies according to the geographical origin of the individuals tested. Thus Reimann and Price (1949) showed that whilst 78 per cent of the residents of Missouri gave a positive reaction the corresponding figures for Illinois and Northern California were 9 to 11 per cent and only 3 per cent respectively. Those regions which yield the greatest number of reactors are also the ones which have the largest number of cases of histoplasmosis.

2 The percentage of positive reactions varies with the presence of pulmonary calcification in the individuals tested. In studying the value of the test with nurses Palmer (1951) showed that whereas 50 per cent of them as a whole reacted to histoplasmin the proportion was 85 per cent in those who had pulmonary calcification. Similarly from Tennessee residents Christie (1940) obtained 87 per cent of positive reactions in those with calcifications whilst in those without calcification the percentage was half as great. Further only 18.8 per cent of those with calcification reacted to tuberculin and the whole population gave only 18.8 per cent of reactors positive to tuberculin.

Olson Bell and Emmon (1941) were however unable to establish a clear relationship between the presence of pulmonary calcification and the extent to which there is positive reaction to tuberculin or histoplasmin.

In any case conclusions drawn from many epidemiological investigations at present support the view that histoplasmosis exists in a mild form very much more widespread than the serious form recognized up to the present and that this mild form is in certain regions responsible for a great many cases of pulmonary calcification previously attributed to tuberculosis. Nevertheless the use of histoplasmin does not permit of diagnosis of histoplasmosis and there are still cross reactions between histoplasma and blastomycetum.

Prior Cole and Torbet (1940) claim however that this test has a specific value in the case of the dog.

Histoplasmosis in Africa

As yet little attention has been paid to this question even though it possesses an interest out of proportion to the number of the cases reported namely — cases 1943 and 1946 (Duncan), 1 case in 1947 (Fries and Deloys) and other cases (Herrman and Arctas). This interest stems from two facts—

- 1 The lesions noted are mainly cold abscesses some of which tend to heal.
- 2 The parasite recovered from the lesion has greater dimensions than those usually given (10μ in diameter instead of 3 to 5μ) and it is usually extracellular.

It is not unreasonable to speculate as to whether this is a distinct species. Catrusi (1941) who made an exhaustive investigation of most of these African strains makes no special comment upon this apart from its poorly marked pathogenicity.

Taxonomy

Moore (1934) erected the genus *Powderm* after having considered the echinulate chlamydospores to be asexual. However de Vriesman who first described them regarded them as no more than ascus like bodies or ascus like cells and it is generally agreed that they are not asexual.

In 1934 Ciferri and Redaelli proposed the family Histoplasmaaceae and reconsidered this question with Virocchi in 1938. These authors believed that the Histoplasmaaceae should be placed near the Nectronomycetaceae (Ciferri and Redaelli 1939) and the Torulomycetaceae (Ciferri and Redaelli 1939) within the larger family of the Adichnecharomycetaceae (Ciferri monod) *sensu ampliore* Ciferri 1940. The Histoplasmaaceae comprise the single genus *Histoplasma* Durling 1906 of which there are three species *H. capsulatum* Durling 1906 *H. furciforme* m. R. Ohta and Mitsuoka 1937 and *H. m. m.* (Shott) Ciferri and Redaelli 1934. The conception of Ciferri *et al.* is based essentially upon the yeast like aspect of

H. capsulatum in its parasitic phase, whereas when cultured the fungus has both a filamentous and a yeast like phase. In spite of objections Ciferri and Redaelli (1918) have maintained their point of view.

Hansenmann and Schenken (1934) having assigned a fungus isolated from a case of histoplasmosis to the genus *Sepedonium* Howell (1930-1940-1941) compared the morphology and physiology of *Histoplasma capsulatum* with various species of *Sepedonium* (*S. chrysospermum* and *S. xyloenum*) with *Streptomyces triseococcum* with *Chlamydomyces palmarum* and with *Mycogone perniciosa*. *Sepedonium chrysospermum* forms aleuric spores identical with the echinulate chlamydospores of *H. capsulatum* and aliphatic spores. *Sepedonium xyloenum* does not form phialospores and its aleuric spores although similar in morphology to the echinulate chlamydospores of *H. capsulatum* are of different origin. *Streptomyces triseococcum* forms phialospores and its aleuric spores are different from the chlamydospores of *H. capsulatum*. *Chlamydomyces palmarum* and *Mycogone perniciosa* form aleuric spores and phialospores which seem to justify the assignment of these two species to the genus *Sepedonium*. Howell concluded that the genus *Histoplasma* constitutes among the *Fungi imperfecti* a distinct genus possessing certain morphological affinities with the genera which he has studied.

From the facts here put forward it appears that the family Histoplasmaeae may be retained for the genus *Histoplasma* but it is absurd to assign this family to the Adelomachromycetaceae which are yeasts for the Histoplasmaeae are filamentous fungi with a yeast like form.

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B. EPIZOOTIC LYMPHANGITIS IN SOLIPEDES

Epizootic lymphangitis in solipeds is a transmissible contagious and spontaneously curable disease caused by *Histoplasma farciminosum* (Rivolta and Micellone 1883) Ciferri and Rida 1914. In the horse the disease and the donkey it produces nodular lesions often ulcerated which spread along the lymphatics and reach the ocular nasal and buccal mucosa. The disease was originally centred on North Africa especially Algeria but from there many cases have reached a world wide dissemination since the First World War (1914-18).

The pathogen often referred to as *Cryptococcus farciminosus* (or *farcimosa*) was long thought to be a protozoan in 1912 Da Rocha Lima assigned it nearer to *Histoplasma capsulatum* suggesting at the same time the fungal nature of the two organisms. Attempts at culture have been made since 1897 by Marcon 1898 by Tschirg 1898 by Barubello and 1906 with a certain amount of success by San Felice but Negre and Boquet in 1918 obtained the first series of cultures and transmitted epizootic lymphangitis of the horse by their use. Some years later Bardelli (1914-1915-1926) in turn isolated the *cryptococcus* inoculated his culture in the horse and even prepared a vaccine. Negre and Boquet and also Bardelli were able to culture the filamentous form of the pathogen by inoculating pus into a fairly simple medium such as Sabouraud with the addition of crushed lymph node (Negre and Boquet) or thymus (Bardelli) from the horse. Upon these media the yeast like forms characteristic of the pathogen in the tissues enlarge and then give rise to mycelial filaments which yield chlamydospores and what Negre and Boquet called external spores. The latter which are undoubtedly

CHAPTER VI

The Levuroses

Introduction

The levuroses are diseases caused by true yeasts. There are two types of such diseases, namely moniliasis caused by *Candida albicans* and torulosis or cryptococcosis caused by *Torulopsis neoformans*. As will be seen later there are certain other species of *Candida* besides *Candida albicans* which possess some pathogenicity, but in practice only *Candida albicans* and *Torulopsis neoformans* are of importance in this respect.

A precise definition of a yeast is not easy to give, though it is generally accepted that the yeasts are fungi which reproduce only by budding. But the ability of certain filamentous fungi (e.g. *Hyaloplasma sporotrichi* or *Blattomyces*) to reproduce by budding under certain conditions either in the saprophytic or the parasitic state renders this definition void. According to Skinner in his recent work, "The yeasts are true fungi of which the habitual and dominant developmental form is unicellular." Skinner thus clearly emphasizes the dominant morphological mode of budding. On the other hand the ability of many yeasts to form a pseudo-mycelium brings them nearer to the filamentous fungi even though the budding mode of formation of the pseudo-mycelial filament distinguishes them from the characteristic germination of true mycelial filament. Lundgren (1941) showed the budding process involves the extension of a tube from the central vacuole to a region of the cell wall which becomes thinner as it forms an excrescence upon the mother cell. Within this excrescence which later becomes the daughter cell the vacuolar tube penetrates and sets up the vacuole of the daughter cell. The two cells separate by a bival constriction at the identical position where the excrescence was formed. When having reached the size of the mother cell the daughter cell separates from it as a new yeast cell capable of producing similar buds which either separate or remain attached. If the daughter cell continues growth in one direction to form a filament which in turn may bud off others a pseudo-mycelium results. The pseudo-mycelium made up of filamentous and rounded elements in an intricate complicated branching system the morphology of which was recorded and sufficient details by Langdon and Talbot (1912) for the mention of the main distinctions. Langdon and Talbot (1913-40) gave the name blastosis (blastosis) to a pseudo-germination process described in 1892 by Roux and Lanoisier. In cultures of *Candida* there is the appearance

creation of the genus *Candida* whilst Stelling Dekker's work (1931) on the sporogenous yeast and the papers of Lodder (1934) and of Diddens and Lodder (1941) on the asporogenous yeast are noteworthy. Lodder and de Vinger (1947) have divided the asporogenous yeasts into two families the Rhodotorulaceae and the Torulopodaceae. The Rhodotorulaceae are asporogenous yeasts which develop a red pigment of a carotinoid nature. These yeasts have neither pathogenic nor fermentative power. Very widespread in nature they are frequent contaminants of culture media inoculated with pathological product. The Torulopodaceae are asporogenous yeasts placed into two sub-families according to whether they form a pseudo mycelium (Mycotoruloidaceae) or not (Torulopodaceae). The latter comprise six genera *Torulopsis*, *Pityrosporum*, *Mycoderma*, *Aloclera*, *Trigonopsis* and *Schizoblastosporium*. Only the genus *Torulopsis* characterized by the formation of a thick capsule include a pathogenic species namely *Torulopsis neoformans* the agent of torulosis or cryptococcosis. The Mycotoruloidaceae comprise three genera *Candida*, *Trichosporon* and *Brettanomyces*. According to Langeron the genus *Trichosporon* which forms a true mycelium ought not to be classed with the yeast; it includes the species *T. beigeli* the agent of the white piedra. The *Trichosporon* species are in any case distinct from those of *Candida* and *Brettanomyces* for they form arthrospores. The *Brettanomyces* species (cf. Curson 1940) are Mycotoruloidaceae distinguished by the oval form of their cell and intense acidification of the culture medium under aerobic conditions. They are of practical interest only to the fermentation industry (*Brettanomyces bruxellensis*, *B. lambicus*). The *Candida* species are Mycotoruloidaceae with the yeast form rounded or oval and the pseudo mycelium forming a complicated structure ornamented with elaborate whorls. Some *Candida* species are pathogenic the most important of these being *Candida albicans*. Diddens and Lodder have distinguished species of which 19 are saprophytes and 1 are pathogenic. The *Candida* species are distinguished from one another by their fermentative action on sugars and the nature of their sugar assimilation. Only the albicans group (*C. albicans*, *C. tropicalis*, *C. stellatoidea*, *C. truncata*) form chlamydospores. These characters distinguish them sharply from other Mycotoruloidaceae.

Skinner (1941) recognises three families of imperfect yeast.

1. The Sporobolomyetaceae which Stelling Dekker and Brettanomyces but which Skinner considers to be imperfect fungi probably related to the Brettanomyces.

The Rhodotorulaceae corresponding to the Rhodotorulaceae of Lodder.

The Torulopodaceae comprising the Torulopodaceae of Lodder and the Mycotoruloidaceae of Lodder.

The Torulopodaceae of Lodder are divided into the Torulopodaceae and the Mycotoruloidaceae. The Torulopodaceae of Lodder are divided into the Torulopodaceae and the Mycotoruloidaceae. The Mycotoruloidaceae of Lodder are divided into the Mycotoruloidaceae and the Torulopodaceae.

3 The Cryptococcaceae corresponding to the Torulopsidaceae of Diddens and Lodder. The Cryptococcaceae are divided into two subfamilies the Cryptococcoidae (Torulopsidoidae of Diddens and Lodder) and the Candidoidae (Microtoruloidae of Diddens and Lodder). The Candidoidae is distributed amongst three genera. Form which produce blastospores only are put in the genus *Candida* or the genus *Brettanomyces*; those which produce both blastospores and arthrospores are put in the genus *Trichosporon* those which produce arthrospores but no blastospores are excluded from the family. They include such organisms as *Crotonchium candidum* (Oudemans).

In the case of imperfect yeast the species is based essentially on such biochemical characters as can be deduced from fermentation studies and the utilization of sugars or of nitrogenous substances. Lançon and Guerra reserve the name fermentation for the anaerobic breakdown of sugars accompanied by the formation of carbonic acid. The term sugar selectivity refers to the capacity of a yeast to metabolize this or that pure sugar. The English terms are gas production (C) and acid production (A). The utilization of nitrogenous substances emphasizes the capacity of yeast to utilize one or another nitrogen compound generally an amino acid as their sole nitrogen source.

The stability of the fermentation characters of yeast has been much discussed. Most theories regard them as labile when transition occurs from phase R to phase S. According to Lançon and Guerra who are amongst the most recent supporters of this view apparent divergencies of result are due to three main causes—

1 The use of yeast strains contaminated by bacteria. These bacterial infections may persist for a long time.

The use of mixed strains. It often happens that a number of yeast strains the colonies from which represent mixture rather than single strain are isolated from a pathological product. Fermentation studies from these give aberrant results unless the mixture should include symphic and asymphic yeasts.

3 The use of impure sugars.

According to Haltrøen (1930, 1938) there are two principal types of enzyme constitution: enzymes formed by microorganisms irrespective of substrate and adaptation enzymes which result from chemical stimulation. The capacity to form adaptation enzymes is transmitted from strain to strain but may disappear if the composition of the culture medium is altered. According to Rhoads (1941) mutation could bring about stable modifications in the formation of enzymes. From this it would seem that there are two causes of variation in fermentation capacity—adaptation and mutation.

Rhoads carried out his experiments with *Saccharomyces*. In medical mycology as hitherto practised the stability of enzymes may be accepted for the determination of *Candida* species provided that the conditions are

indicated above namely purity of the strains and purity of the sugars are scrupulously respected. George and Plunkett (1948) investigating ten strain of *Candida albicans* kept for 3 to 4 years in the laboratory noted morphological modifications in six of them but not one showed the slightest alteration in the fermentation characters.

A THE MONILIASES

Definition

The name moniliasis is given to acute, sub-acute or chronic infections caused by yeasts belonging to the genus *Candida* and chiefly to the species *Candida albicans*. The skin and the mucous are most frequently involved.

The term moniliasis is used in current publications and should be retained. As Skinner has noted (194) it would be regrettable if modifications in the systematics of the genus in question should once more determine a change in the nomenclature. The older nomenclature of two kinds either it is derived from the taxonomic names of the causal agent and one thus refers to odious torulosis, cryptococcosis, mycetozulosis, saccharomycosis or it involves dermatological terms of a primarily topographical nature e.g. thrush, perleche, onychia, paronychia, vulvovaginitis. These terms are usually corrected by the addition of the name of the pathogen responsible: onychia à *Monilia albicans*.

Historical

In 1830 Langenbeck recognized the presence of fungi in thrush. Almost simultaneously (Ruhé (184) who had studied this disease for more than a year called the fungi of thrush asphthophyte. Rolan (1843) named the pathogen *Oidium albicans*. The term *Oidium* was unfortunate enough for it is applied to the imperfect form of Erysiphaceae (ascomycetes, pycnomycetes) and cannot be retained. It may be thought apposite to recall here that Pasteur's first paper on alcoholic fermentation which formed the basis for all subsequent studies on fermentation was published in 1857. In 1858 Quinquaud first changed the name *Oidium albicans* to *Syngasteria roborans*. Quinquaud having recognized the non validity of the name *Oidium* several workers consider the preference for *Syngasteria* to have priority over that of *Candida* which was accepted in 1939 by the Third International Congress of Microbiology. In 1888 Plant named the pathogen *Monilia candida* and in 1890 Zeller described *Monilia albica*. This last appellation prevailed in the literature up to the work of Berkhout in 1928. *Monilia* cannot however be accepted for it is applied to the imperfect form of *Stromelia* (ascomycetes and comycetes) the lent brown rot of fruit.

Since the beginning of this century interest in monilia is increased a strong stimulus from the work of Castellan (Castellan 1938) who not only described bronchomoniliasis but he was the first to isolate the

Accepted by Langenbeck as the first name for the fungus
[Fungi] [Fungi] [Fungi] [Fungi] [Fungi] [Fungi] [Fungi] [Fungi] [Fungi] [Fungi]

pathogenic yeasts on a fermentation basis. In 1903 Berkhout then created the genus *Candida* which has since been retained and separated it from many other fungi of similar morphology.

In 1909 Rava and Rabreau in an eminent work established the lemma and drew attention to a product of this group which they called leucon.

In 1931 Benham through original work and an important critical review of the literature showed that a classification based on purely morphological or purely biological considerations was insufficient to distinguish the *Monilia* from one another and that most of the strains isolated from pathological materials belonged to the species *C. albicans*. Langeron and Telle in 1911 tried to distinguish between six morphological groups within the genus *Candida* but in 1938 Langeron and Carra denounced this basis of separation as inaccurate and produced their work on the fermentation reaction of the yeasts of the genus *Candida*.

The work of Lorkh (1934) and of Dodden and Lodder (1941) exerted great influence upon the classification of the anamorphous yeast.

Importance and Geographical Distribution

Monilia is a relatively frequent mycose of world wide distribution. Though it is found at all ages in both sexes and without racial distinction certain predisposing factors are recognized. Malnutrition, scours, moniliasis of the mucous membranes, beauty that of the fold and diabetic and pregnancy probably encourage the development of vulvovaginitis caused by *Candida albicans*. Therapeutic baths, damp dressings, frequent immersion of the hand in water and the handling of fruit, all frequently though but favour the disease. Fifty cases of endocarditis were reported in drug addicts who regularly subjected themselves to subcutaneous injections; the role of the injection or of the drug is unknown.

Etiology

Candida albicans is normally present in man. Its incidence increases in the midwife with age and may increase considerably in certain pathological or therapeutical condition. Administration of antibiotics in particular seems to increase the number of yeast in the sereament at the same time diminishing that of bacteria.

Todd (1937) whilst searching for *C. albicans* in the mouth and the throat found it in 42 per cent of 50 persons more than 50 years old, in 7.2 per cent of 264 young students and in 20 per cent of 72 adult women. Out of 614 young people he isolated it in 8.7 per cent of 998 males and in 17.7 per cent of 316 females. Of 1000 persons under observation 140 yielded *C. albicans* and 7 gave non-determined yeasts. 93 per cent of these were males and 18 per cent females. In addition this same worker noted agglutination of *C. albicans* by the serum of 22.8 per cent of 1150 normal persons, 30.4 per cent for women and 16.7 per cent for men.

Castellani observed a certain incidence of bronchomycosis amongst tea tasters in Ceylon. Törnell (1916) blamed the dust from corn threshing

for certain pulmonary alterations attributed to yeast. Nilby and Norden (1949) tried to demonstrate the presence of *C. albicans* in the atmosphere. 600 petri dishes exposed to the air in a town did not give a single colony of the organism in question. The same result was noted for 110 exposures in houses or hospital. However, petri dishes placed on bedside tables of patients with *C. albicans* in their buccal cavity have yielded some colonies of the fungus. Nilby and Norden have also searched unsuccessfully for the organism in dust from the lungs and report Tornell's hypothesis. In the buccal cavity of healthy men they have found *C. albicans* in 30 per cent of their cases and in 6 per cent of patients with pulmonary disease. Their conclusion is that *C. albicans* is rarely found outside the human body.

Skinner (1947) however, had already emphasized the rarity of the strains isolated outside the human body and he noted from the collection examined by Diddens and Lasker that only two strains came from soil. According to Skinner it is a mistake to regard *C. albicans* as a ubiquitous spread in nature. One would expect the organism to be present on dairy produce and he has on several occasions isolated it from Camembert cheese. Again he has drawn attention to the work of Vrak Phaff and Vaughn (1941) who isolated it from date and of Vrak and McClean (1940) who isolated it from grape.

Candida albicans is thus a yeast which is frequently isolated from man, healthy or otherwise and also from dairy produce and fruit. It is apparently more frequent in women than in men and it is more frequently isolated from patient with lung trouble than from healthy individuals. It is probably a saprophyte of wide geographical distribution which under particular conditions can become pathogenic.

Pathogenic Agent

The *Candida* species are yeasts which grow easily at laboratory temperatures upon numerous media and especially upon Sabouraud's percent glucose medium. Inoculation at a point on agar on which even moist creamy growth appears within 48 hours which has been filamentous rather quickly into the liquid if the agar is inoculated. Inoculated in a test tube it also grows at a point and is easily lifted from the surface with a pipette into the agar filament appears on either side of the streak in the tube. The aspect of *Candida* colonies are with the typical filamentous growth. Inoculation yields a more or less regular colony which is white at the height of its growth. We define a normal colony as being larger than *C. albicans* as one which is said to grow the whole of the medium, in the quantity of medium filled to it and the surface of the tube. It is often in the culture alone it is sufficient to produce the culture in the petri dishes and Erlenmeyer flask are unnecessary.

Important has been attributed to the characteristics of the filamentous on liquid as well as solid media. When the filamentous growth is both it usually produces a surface turbidity and the filamentous growth is the

bottom of the tube. As the development in the form of hyphae and their growth often keeps the liquid medium clear they also often produce a concentric ring. At the periphery of the tube which is important in attachment. If the ring develops towards the centre it eventually forms a solid which is described as a mass if it develops at the bottom of the tube in small fragments and membranous if it falls with difficulty and in larger portions. As I only occasionally see young cultures of *Candida Crataei* how they form a mucous film within the fifth day.

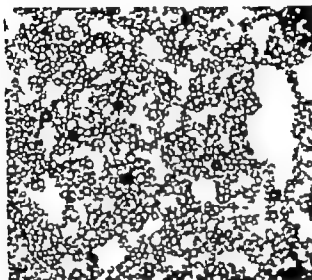


Fig. 43

C. Crataei. (Cultured in agar) (Cultured in agar)

A fragment of a colony mounted in a drop of water shows a large number of round elliptical cells 2 to 4 μ in size many of which are bent to buds. If the colony is slightly killed or if a fragment of agar containing filaments is carefully prepared by hand and slip a short time out the mode of filament formation is characteristic of *Candida glabrata* can be seen. The arrow spores cannot be distinguished on the basis of the various morphologies recorded by Langeron and Taber.

The most important morphological character is undoubtedly the formation of chlamydospores by the *Candida* species of the *libra* group (*C. libra*, *C. crataei*, *C. stellata*, *C. glabrata*). The chlamydospores are large spherical cells 8 μ or more in diameter refringent double contoured and which usually surround the cell of the filament. According to Kligman (1940) the condition in which the

development (i) a poor culture medium (ii) reduced oxygen pressure and (iii) a low temperature. Culturing at 37°C clearly inhibit the formation of chlamydospore. Long ago Benham demonstrated the value of using agar with maize flour for their production.

Though they can arise upon undifferentiated hyphae the chlamydospores usually form upon a swollen articulation of the mycelium which was termed a protochlamydospore by Langeron and Cuerra (1939). The protochlamydospore is full of very condensed protoplasm, is acid fast and can itself change into a chlamydospore or empty its contents into another chlamydospore which may arise from it. Protochlamydospores most frequently yield single terminal chlamydospores but bunches of three or four or both chain may develop from them.

It has long been observed that the macroscopic morphology of yeast colonies varies with transplantation. Colonies which are creamy at their first isolation often become membranous after a more or less lengthy period. By analogy with bacteria the expression phase S is given to the creamy phase and phase R to the membranous phase. These dissociation or variation phenomena in *Candida* colonies have given rise to a great deal of research since the time of Senné's original observations in 1890. Phase S differs from Phase R in the following respects—

1. There is predominance of the yeast phase over the mycelial phase. Whereas the creamy colonies practically only contain yeast cells about 1 μ in diameter the membranous ones chiefly consist of pseudo mycelium with elements of greater dimension than those of the creamy phase.

In liquid media phase R always forms a veil whilst phase S forms at most a ring and exceptionally a mucous veil.

3. The transition from phase S to phase R reduces and even the disappearance of the pathogenicity.

Yet other forms have been distinguished such as form I (ketal form) (MacKinnon 1940) which tends to disappear if it is not frequently transplanted. Langeron and Cuerra (1941) have correlated with these phase variations the appearance of dark and characters in certain yeast colonies notably of the genus *Candida* the characters contain small rounded cells and the dark ones very large cells.

It has been observed that in an abundant culture medium which is well aerated and rich in assimilable carbohydrate the S form predominates and tends to maintain it. If Phase R appears with diminishing oxygen if the culture medium is poor (maize agar, potato extract or potato) or if the culture is set up in a liquid medium giving partial anaerobiosis. Acidity favours phase S and alkalinity phase R.

It is important to note that the transformations are not reversible and that it is possible to pass directly from phase R to phase S and that whatever morphological modifications appear in the yeast the fermentative properties remain unaltered.

The genus *Candida* has numerous synonyms of which the following are the most important—

produces filament which run over the surface of the agar and the adult colony has truncated per *C. albica* var *tried* prod. as a mucous veil in liquid med. *C. krusei* forms a thick veil in liquid media. Biological characters may also be considered in conjunction with these. *C. tropicalis* and *C. albica* are the only *Candida* pathogenic for the rabbit. *C. albica* (and its group) *C. tropicalis*, *C. krusei* and *C. guilliermondii* are probably the only ones pathogenic for man and *C. albica* is by far the most frequently encountered.

Symptomatology

The moniliasis are localized or generalized mucocutaneous infections and much more rarely involve the viscera.

A Mucocutaneous Moniliasis

1 **Cutaneous Lesions** The most frequent form is intertrigo. The lesion takes the form of a reddish patch following vesicle formation. Frequently there is running sore and fissure development at the bottom of a fold. Fournier and Rabeau (1936) consider that the contour of the patch is characteristic: it is finely notched irregular shredded edged with a thin epidermal ridge whitish detached to the extent of about 1 mm. The lesion starts or itches violently. When situated in an inguinal fold it rarely involves the scrotum and the penis but frequently the intergluteal region. In women it often spread to the labia majora.

Other frequent localizations are the infra mammary folds, the axillae, the umbilicus and the interdigital folds of the fingers and toes. In the hands in 50 per cent of the cases the lesions are limited to the third interdigital space and are never found in the first space.

From the interdigital spaces of the feet the lesions may travel up the back of the foot forming circumscribed erythematous and vesicular patches.

2 **Onychia paronychia** (*albica* frequently) involves the perungual fold. Amongst American workmen handling fruit the disease may be regarded almost as occupational. The skin above the ungual fold becomes red stretched tumefied and painful and slight pressure may cause the exudation of drop of pus. Onychia is commonly described if the lesions were due to the presence of *C. albica* but authors are usually discreet about the exact description of the lesions. Lewis and Hopper (1948) neatly discriminate between the onychia of moniliasis and that caused by dermatophytes. It is at once apparent they write that the cardinal signs of tinea unguium namely crumbling yellow colour and loss of lustre together with lack of paronychia readily distinguish the two conditions. Fournier and Rabeau (1936) however claim that the nail becomes opaque and thickened raised by a hyperkeratotic mass and that it is often impossible to differentiate it from a nail attacked by a trichophyton.

We believe that the onychia associated with *C. albica* is the result of trophic troubles caused by the paronychia. The nail does not appear

to be invaded by the yeast Vanbreughem (1930) has in any case shown that *in vitro* *C. albicans* is incapable of attacking keratin.

3 Mucocutaneous Lesions Perleche which invades the commissure of the mouth and may spread to the cutaneous lining may be regarded as a mucocutaneous form. The whitish blotches which cover the red margins of the lips become progressively erythematous squamous.

Because of the frequency of cutaneous involvement vulvovaginitis may equally be considered in this group. Frequent in pregnant or distal women it is characterized by copious white emission vulvar pruritus and often by erythematous squamous lesion of the outer vulva and the inner surface of the thighs.

4 Mucous Lesions Vulvovaginitis uncomplicated by cutaneous lesions comes together with thrush into this group. There is a stomatitis which may or may not leave the tongue intact characterized by a white lustreless coating which adheres to the mucous membrane. It is found especially in individual suffering from malnutrition.

5 Generalized Lesions Spread of a moniliaemia to the mucocutaneous lining is exceptional. When it appears all the symptoms already given in a more or less mixed form may be detected. Seborrhoeic involvement with folliculitis decalvans appears to be frequent in these cases (cf Gray Lambelin and Vanbreughem 1930).

Two forms may be distinguished one of earliest infancy and that of later infancy. The latter which is rarer is also more resistant to treatment. The first form may pass into the second. In adult generalized moniliaemia of the skin has been reported following protracted therapeutic baths and moniliaemia have been extended by the use of damp dressing.

B Visceral Moniliaes

The best known is bronchomoniliaemia the first information having been provided by Castellani in 1910. It is however very much less frequent than this worker at first thought to be the case. Diagnosis must be given with extreme caution. The sputum mucoid and gelatinous is usually colourless sometimes streaked with blood the cough is refractory.

Pulmonary moniliaemia has also been described of varied and local report and as a generally grave condition. Many of the cases described however are really secondary infections of old tubercular disease bronchial dilations and cancerous lung.

Five cases of endocarditis have been reported in drug addicts who injected them exclusively. In four of the cases *C. parvulus* was isolated and in the fifth *C. guilliermondii*.

C Granulomas caused by *Candida albicans*

Moore (1918) and Hauser and Latham (1930) described granulomas produced by *C. albicans*. In Moore's case there was a lesion in the larynx.

The granuloma has been reported in the skin of the nose and in the ear. The granuloma remains unaltered.

of the hand 9 cm by 7.5 cm with a raised margin and a granulated centre. Histopathologic examination revealed rare yeast forms in the tissues. Hauser and Rothman in addition to their actual case report 13 cases in the literature. The lesions are certainly very rare.

Histopathology

When there are granulomatous lesions yeast cells filamentous giant cell and epithelioid cells are found in the tissues.



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Treatment

Cutaneous and mucous moniliasis may usually be treated fairly easily. One of the most effective treatments for cutaneous moniliasis is to apply a 2 per cent alcoholic solution of eosin. Whitfield ointment and Castellani paint may also be used (cf. Dermatophytes). Onychia and paronychia are treated by bathing with 1/4 000 potassium permanganate or applying in the form of wet dressings followed by 1 per cent gentian violet.

Vulvovaginitis yields easily to borate or bicarbonate injections. Where diabetes is present this naturally has also to be regulated.

Thrush and Perleche react favourably to application of 10 per cent glycerine borate. The general state of the patient has often to be improved as it is often an important factor in the progression of the disease.

The broncho and pulmonary moniliasis is treated with potassium

A subcutaneous injection may cause an abscess in 48 hours.

To a less extent *C. tropicalis* is pathogenic for the rabbit. The other species are not.

Allergy and the Levurins

Antibodies (precipitins, agglutinins) are readily demonstrated in the serum of healthy individuals or patients; these reactions have only group specificity and are inconclusive from a diagnostic point of view. According to Todd (1937) the agglutinin titre does not usually go beyond 1:160 but Conant *et al.* claim a much higher titre of 1:400.

Pavant and Rabreau (1928) showed that there could appear generally on the occasion of growths rising at the level of initial lesions and mainly at the time of relapse or following a levurin injection, lesions which they called levurids. These almost invariably symmetrical lesions develop on the arms, forearms, sides, buttocks, the inner surfaces of the thighs, the face and may be localized or generalized. They comprise salmon pink, brick red maculose efflorescences at a level at which the skin is more or less infiltrated; they are rarely pruriginous (Pavant and Rabreau 1936).

The levurids have the general characters of skin (dermatophyte infections); they are sterile and heal by treatment of the initial focus.

Pavant and Rabreau prepared a substance which they called levurin (monilium oxyomycesin) by a method similar to that used for trichophytin. Injected intradermally, ant. patient with moniliae, it may produce—

1. A local reaction similar to that which follows trichophytin injection: i.e. immediate reaction of the urticarian type, late reaction of the tuberculous type.

A focal reaction in the form of an eczematiform growth at the margin of parasitic constituent.

3. A local reaction on a site previously injected with levurin.

On the other hand, a reaction which has died down can be re-activated by a revival of the parasitic foci.

The intradermal reaction to levurin has no specific value; it is a group reaction. Its absence from a moniliasis patient would be abnormal but cases of anergy are recognized. Its presence in a healthy subject does not justify any conclusion.

There are no cross reactions with trichophytin, sporotrichin or tuberculin.

Moniliae in Animals

Incidentally reviewing the literature while dealing with mycetozoa, parasites in hedgehog, Talice (1912) drew attention to cases of thrush in the hen (Neuman), the lamb (De Lafond), the cat, the dog, the turkey (Blanchard), in *Circopithicus patas* or *ruber* (Thury, 1913) and in the calf and the foal (Brumpt). Catanei (1925) noted Monilia on the tongue of the

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B TORULOSIS OR CRYPTOCOCCOSIS

Definition

This disease which has several synonyms such as *Torula meningitis*, yeast meningitis, Busse-Buschke's disease, European blastomycosis is a chronic one with the essential symptomatology of an abscess meningitis accompanied by violent headache and considerable hypertension of the spinal fluid.

Historical

The aetiological agent was first isolated by Busse in 1896 from a subperiosteal lesion of the tibia of a woman. The pathogen was in 1910 called *Saccharomyces hominis* by Vanthman.

In 1894 however Binfelice isolated the same organism from fruit juice in Italy and called it *Saccharomyces neoformis* this *neoformis* must be retained whatever the genus to which the organism concerned in torulosis should be assigned.

In 1916 Stoddard and Cuthrie first isolated the pathogen from the brain and marrow of a man. They called it *Torula histolytica* for they believed that they had an instance of tissue destruction caused by a yeast capsule.

Redaelli in 1931 assigned the pathogen to the genus *Torula* when according to the work of Lodder it should apparently remain. The name

T. histolytica was proposed by Lodder and an Hg (T) was used to denote it as *Torulopsis* for some special fermenting yeast as it has 14 amino acids here whereas the pathogen of torulosis is genus *Cryptococcus*.

of the organism should thus be *Torulopsis neoformans* (Sanfelice 1894) Pedaeili 1931

Todd and Herrmann: 1936 described the formation of a monosporeous asexual and called the yeast *Debaryomyces hansenii*

In 1937 Pedaeili Ciferri and Cardano 1 tried to have confirmed the observations of Todd and Herrmann but regarded the epithet given by their compatriot Sanfelice as preferable so that according to them the agent of torulosis was *Debaryomyces neoformans*

Most of those who have dealt with torulosis have however been unable to confirm the observations of Todd and Herrmann and of Pedaeili *et al* It is thus pertinent to inquire whether there are several agents of torulosis or only one agent able to reproduce by means of asexual spores under circumstances as yet undefined

In Western Europe the first case was described in Holland by Steenker (1934) the second was apparently the Belgian case of Braucher Scherer and Thomas (1939) though these workers did not isolate the pathogen and their diagnosis is purely histopathological the third was the French case of German and Morvan (1939) followed eight years later by a fourth (or second French case) of Debré Lamé Vach Crumbach and Normand (1946)

Importance and Geographical Distribution

Torulosis is a cosmopolitan disease of which there have been about 170 cases described up to the present Though the infection was first described in Europe only 3 French 1 Dutch and 1 Belgian case have as yet been recognized Most of the instances have emanated from the United States and Australia takes an important place with some thirty cases

No race is particularly prone to torulosis and no predilection for any particular profession has been noted Most cases have been found between the ages of 20 and 60 with men twice as frequently attacked as women

Etiology

Torulopsis neoformans occurs naturally as a saprophyte and indeed Sanfelice in 1894 isolated it from fruit juice It has since been found in wasps nest on grass the bodies of insects buttermilk and turned milk The strains isolated from sources such as these are not always pathogenic however Rabinowitch experimenting with 40 strains under laboratory conditions found only 8 to be pathogenic though *Torulopsis* isolated as a pathogen from human sources always proved to be pathogenic for mice

The method by which this yeast gains access to man is obviously important It is frequently held to be via the respiratory tract but usually on inadequate evidence Wade and Stevenson (1941) attempted intranasal instillation in mice and were able to produce some rhinitis and in one case pulmonary lesions but no cerebral lesions Yeast cells were however retrieved from the top of the nasal cavity upon the septum and

the turbinate bone (cornet) and in the sinuses. In several cases lesions occurred as far as the cribriform plate but in no case was the brain affected. The mucous membrane of the nose eroded and formed large cystic masses but prior to the appearance of this erosion the infiltration of yeast cells could be found for a long distance below an apparently intact mucous membrane.

Debre Lamy Leblou Nick Crumbach and Normand (194) claimed that in their case the penetration was via the skin. In fact in their patient a small cutaneous tumour situated upon the chin and rich in yeasts appeared whilst his symptomatology was that of Hodgkin's disease and shortly before his death from a *T. neoformans* meningitis.

Experimentally as will be seen all routes of inoculation may be fatal in the production of cerebral lesions for the brain and especially the meninges are the essential regions of attraction for these yeast.

Pathogenic Agent

The view of most of the classical and modern workers is that *Torulopsis neoformans* is a non filamentous yeast which does not form spores. It reproduces by buds which have the special features of being mostly single and of having a very narrow neck. The cells are perfectly spherical and surrounded by a thick mucilaginous capsule the thickness varying with the strain the age and the culture medium. The cell diameter is 4 to 6 μ that of the capsule 1 to 2 μ in culture.

In tissue the cell varies considerably in form and diameter some cells are 2 to 3 μ and others 10 to 15 μ . They are usually round but some of them are ovoid whilst others as Wade and Stevenson (1941) have expressed it resemble a deflated rubber ball half of which has sunk into the other.

Even when cultured on agar slides no filamentation appears.

Torulopsis neoformans is easily cultured on all the usual media and a very abundant growth can be obtained on glucose agar within 48 hours at laboratory temperature. On this medium the colonies are moist shining cream whitish at first then slightly brownish. On a blood medium they have a dry appearance. They grow well on Rautin where they develop flakes at the bottom of the tube. In percent glucose broth they develop fecally at the bottom of the tube without disturbing the medium. In a medium which has ethyl alcohol as the only source of carbohydrate they develop moderately.

Torulopsis neoformans does not ferment sugars but it seems likely that several of them in acidifying the medium. It plus malt sucrose but not galactose or lactose.

By Beijerinck's auxanographic method it can be seen that it utilizes glucose fructose mannose galactose sucrose malt and lactose ammonium sulphate asparagine urea peptone but not putrescine nitrate.

Todd and Herrmann (1946) first described a sexual cycle for *Torulopsis neoformans* and this was confirmed by Edwards (1950) and (1951) and

(1937) According to these workers *T. neoformans* on a maltose or glucose Sabouraud medium reproduces by budding for five or six weeks but as soon as the medium dries up two types of cell appear having thin and thick walls respectively. Both of these cell types can bud but if they are transferred to van Tieghem cells in broth the thin walled cells put out a tube towards those with thick walls. The latter produce only slight protrusions which join the tubes from the thin walled cells the contents of which empty completely into the thick walled cells.

After this fusion the central mass which Todd and Herrmann call the spheroid surround it. If with two membranes the outer one of which is undoubtedly the thick wall of the mother cell. The spheroid is then ejected into the medium and begins to bud.

Todd and Herrmann reserve the name *Cryptococcus hominis* for the non sexual phase and call the sexual phase of the pathogen *Debaryomyces hominis*. Redaelli, Ciferri and Giordano apply the generic name *Debaryomyces* but the specific name *neoformans* so that in their view the organism becomes *Debaryomyces neoformans*.

Lodder and de Vrijer (1947) noted that if these facts are confirmed a mode of ascospore formation is involved which is completely different from that encountered in the other ascosporous yeast.

A number of research workers have directed their attention to the nature of the capsule of *Torulopsis neoformans*. Hlgman (1947) showed that the capsule is essentially composed of polysaccharides but he was unable to produce in the rabbit either agglutinins precipitins or antibodies producing complement fixation either by injecting the whole cell the cell deprived of its capsule or the capsule alone. The essential features of Hlgman's technique for obtaining the capsular substance are as follows.

An emulsion in distilled water from a two week old culture grown on potato agar with dextrose is centrifuged and run into 500 ml flasks. The centrifugation is repeated four times taking up the residue each time with distilled water. The last residue is heated for 25 minutes at 55°C in 0.4% hydrochloric acid. This kills the yeast cells and dislodges their capsule. The same result can be obtained by warming the emulsion for 12 hours at 55°C. The decapsulated cells are removed on the centrifuge and the supernatant liquid neutralized with soda. This is followed by the addition of 10 per cent by weight of sodium acetate and 3 volumes of ethyl alcohol which produces an abundant white precipitate. After refrigeration overnight the solution is centrifuged and the residue taken up in distilled water. An opalescent solution is formed which is precipitated by 2 volumes of ethyl alcohol and 10 per cent sodium acetate. After three reprecipitations a protein free solution is obtained. The burrette Millon and trichloroacetic acid reactions are negative. Lugol's iodine reaction confirms the absence of starch. Fehling and Benedict's test the absence of reducing substances. Molisch reaction is on the other hand strongly positive.

Drouhet and Segretain (1950) claimed that it is possible to dissolve the capsules of *T. neoformans* by submitting these yeast to the action of hyaluronidase.

Symptomatology

Torulosis is essentially an aseptic meningitis of a chronic evolution characterized by violent and persistent headache. The clinical symptomatology depends upon the localization of the lesions and their importance. Generally the symptoms develop progressively and commence with intermittent frontal headache but the onset may be fulminating as in the case of Debré *et al* (1946-1947) where there was a sudden stroke followed by death 15 days later. Amongst the symptoms noted are dizziness, stiffness of the neck, hemiplegia, paraplegia and frequently deteriorating eyesight. This is either mechanical or visual. ptosis, nystagmus, strabismus, papillary stasis and papilloedema. Vomiting is often noted. Troubled sleep and mental disturbance are frequent. Depression, disorientation, apathy, agitation, irritability and delirium have been noted.

In about half the cases the symptoms are localized in the meninges and the encephalon. In a third of the cases however there are pulmonary complications and there are some cases of pulmonary torulosis from the start. These lesions are vague and indefinite and never permit of a specific diagnosis. The symptoms are those of a subfulminant infection of slow development: sputum, rare or absent, sometimes blood-stained. The pulmonary lesions are usually diffuse and bilateral but are sometimes localized at one side in an upper lobe. In the Netherlands case of Steegers (1954) the pulmonary lesion complicated in any case a torulic meningitis was fistulized.

Besides the lesions of the nervous system and of the lungs, *T. neoformans* may occur in the kidneys, spleen, suprarenals, liver, bronchial lymph nodes, subcutaneous tissue, bones and skin. The pathogen has been found in dorso-lumbar and inguinal abscesses and in nasopharyngeal ulcers.

Cill (1954) and Weiss, Perry and Shesky (1955) reported two cases of infection of the orbit leading in the second case to a mycotic infection of both eyes.

All authorities insist on the frequency of the coincidence of torulosis and Hodgkin disease or malignant lymphogranulomatosis which is reckoned at more than 10 per cent. What reason are there for this coincidence? The explanations offered may be put into three categories: (i) the coincidence is purely coincidental; (ii) involvement of the lymphatic system by Hodgkin's disease facilitates invasion by *T. neoformans*. This conclusion was reached by Debré *et al* (1946-1947) in the following case in which the lymphogranulomatosis long preceded the appearance of the torulosis which started with a cutaneous accident. A biopsy which preceded this accident had shown that the lymph nodes were indeed affected with Hodgkin disease but free from torulosis; (iii) A third

theory (Kligman and Weidman 1949) would have it that certain cases of Hodgkin's disease may be a consequence of torulosis. By injecting capsular extracts of *T. neoformans* these workers were able to produce a reaction of the lymphatic system which however they did not consider to be characteristic of lymphogranulomatosis.

Histopathology

The whole of the histopathology of torulosis is dominated by the absence of reaction, but in the long run the compression itself ends by producing chronic inflammatory reactions of a predominantly lymphocytic type. Wherever infiltration has occurred the pathogen is in evidence surrounded by its enormous capsule and imparting gelatinous consistency to the invaded tissues. The parasite may or may not be surrounded by lymphocytes and giant cells which possibly engulfed it. In meningitic cases the parasite is to be found in the meninges, the arteries, the perivascular spaces and in the walls of certain arteries outside the internal elastic membrane. The size of the cells is very variable.

Prognosis

In the great majority of cases prognosis is fatal death occurring from 6 to 12 months after the appearance of the first symptom.

Treatment

There appears to be no form of treatment which is effective or which even influences the course of the disease in any way. In fact decompressive lumbar puncture seems to be the only therapeutic method capable of relieving the patient. Shapiro *et al* has performed 133 successful punctures on the same patient.

Navy and Pawan (1941) tried out 1:10,000 solutions of acriflavine and injected 4 ml of this each day after having removed 60 ml of the spinal fluid without success. Conant *et al* recommend the use of sulphadiazine which must be administered so as to maintain a level of 8 to 12 mg for each 100 ml of blood for several weeks after the disappearance of all symptoms. It is noteworthy however that Jones and Hirsch (1945) have found no evidence of inhibition of development *in vitro* with concentrations of 0.01, 0.1 and even 1 per cent sulphadiazine nor even with penicillin (dosage not given). Wourmel *et al* have observed a quite normal development of *T. neoformans* upon Sabouraud medium with 2 per cent glucose and containing 500 units of crystallized penicillin per ml.

Potassium iodide, gentian violet and thymol have also been used without success. Beck and Voyles (1946) tried the action of potassium iodide and sulphadiazine separately or together upon dogs, guinea pigs and rabbit infected with *Torulopsis neoformans*. No therapeutic action could be attributed to these substances.

Kligman and Weidman (1949) investigated the fungistatic activity of

a very large number of products and concluded that not one of them active *in vitro* is active *in vivo*.

Differential Diagnosis

Torulosis has been confused with tubercular meningitis, cerebral tumour and abscess, lethargic encephalitis, dementia paralytica and lymphocytic choromeningitis.

Mycological Diagnosis

This involves (i) Search for the presence of yeasts in the tissues, pus and spinal fluid (ii) Culture of the pathogen and (iii) Animal inoculation.

1 Microscopic Examination

Search for *Torulopsis neoformans* is most easily carried out by placing a drop of pus, a fragment of cerebral tissue obtained from trepanation or a residue of spinal fluid into a drop of Indian ink. Examination is carried out either under a cover slip or after drying the ink film. The yeast appear as rounded cells 3 to 4 μ in diameter, often in the process of budding, surrounded with a capsule which may be enormous and may reach 50 to 60 μ .

In the spinal fluid care must be taken not to confuse the organism with the blood cells.

2 Culture

Typical colonies can be obtained in 4 to 48 hours upon Sabouraud's medium with glucose. Animal inoculation of the culture permit of reproduction of the human lesion and recovery of the cell with the characteristic large capsules.

3 Experimental Inoculation

The most sensitive and frequently used animals are the mouse, rat and guinea pig. The rabbit is not so suitable. In the hands of Dabry et al (1940-1947) the sheep, dog, monkey (*Macaca*), pigeon and hen remained insensitive.

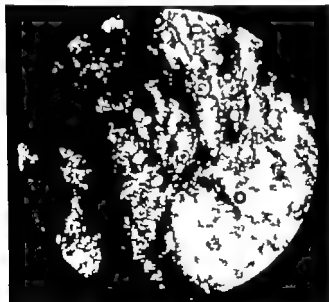
In our opinion the easiest and quickest method for diagnosis is intra cerebral inoculation of the mouse with 0.05 ml of a lightly opaque emulsion of a culture of *T. neoformans* in physiological saline. In our experiment this emulsion contained nearly 3,000 yeast cells per cubic millimetre and its optical density corresponded with the No. 1 tube of Macfarland's nephelometer.

After having slightly anesthetized the mouse with ether the quantity indicated (0.05 ml) is injected with a short needle mounted on a tuberculin syringe into the posterior quadrant of the brain outside the median line. In most cases the mice tolerate this procedure well. Death occurs in 5 to 8 days. In animals which succumb most quickly only cerebral tissue containing for the most part large numbers of cells surrounded with very



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thick capsules will be found. In animals dying towards the seventh or eighth days will be found lesions of the lungs, liver and spleen. A striking feature is the somnolent state of the inoculated animal. They would almost be thought dead from the forty eighth hour after inoculation but if they are handled a little it becomes obvious that they retain considerable reserves of vigour. Further they emerge spontaneously from their lethargic state to feed.

Segretain and Drouhet (1947) carried out subcutaneous, intraperitoneal, intravenous and intracerebral inoculations of mice and reported that death occurred in 6 to 23 days. Intravenous inoculation produced death most rapidly whilst the subcutaneous method was the slowest. These workers considered that the infection always became generalized and cultures from heart blood were always positive. With subcutaneous inoculation a gelatinous mass appeared at the point of injection. Peritonitis resulted from intraperitoneal inoculation, a covering membrane of *Torulopsis* enveloped the spleen and kidneys.

Intraperitoneal injection kills the guinea pig in 45 days. Generalization does not occur but there are lesions of the central nervous system with progressive paralysis of the hind quarters accompanied by tonic clonic contractions before death. In the rabbit intravenous injection does not produce lesions (Segretain and Drouhet 1947, Helyman 1949).

According to Weiss Ferry and Shekely (1949) inoculation of a 0.1 ml emulsion of a 4 hour culture with an opacity corresponding to the No. 1 tube of the MacFarland nephelometer into the anterior chamber of the rabbit eye produces particular kind of lesion. Within 5 to 7 days there appears congestion of the sclerotic and lateral opacification of the cornea. The lesion had to keratitis intermedia and total blindness (fourteenth to seventeenth day). Rosette shaped masses (as the authors call them) appear in the anterior chamber of the eye consisting of a central cell surrounded by a single row of polymorphous and lymphocytes. These rosettes are distributed on the anterior surface of the iris and the posterior surface of the cornea. This was confirmed by Helyman and Weidman (1949).

The spinal fluid may be clear, disturbed, greyish or gelatinous. It precipitates on addition of 0.5 cm of water. There is usually but not always an increase in cell number (from 400 to 4000) and a general leuko-cytosis predominates. The colloidal gold curve is variable but often shows a meningitic or syphilitic reaction. The sugar content may be much lowered. The Bordet Wasserman reaction is negative.

Torulosis in Animals

Brothman described torulosis in the horse in 1940. The lesion was localized in the lung. In 1944 Weidman and Hatchiff reported cases in a leopard the first case recognized from 10000 consecutive autopsies made in the Philadelphia Zoological Park. The first symptoms appeared 5½ years after the acquisition of the animal and the illness persisted for

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CHAPTER VII

The Mycetomas

Definition

The name mycetoma is given to tumour like masses produced by various fungi and characterized by the development of tumours, sinuses and granulations. Actinomycosis caused by *Actinomyces visus* already dealt with once more appears in this category. However the term mycetoma is usually applied to mycotic tumours appearing in the foot and known as maduromycosis or Madura foot. Many different agents are responsible for mycetomas and they belong to genera far removed from one another. The term mycetoma embraces all tumours of fungal origin whatever the systematic position of the organisms concerned, the maduromycoses are mycetomas produced by various fungi apart from those caused by actinomycetes.

Chalmers and Archibald have made a useful distinction between the true mycetomas characterized by the presence of granules and the mycotic tumours devoid of granules which they call para mycetomas. On the other hand they call pseudo mycetomas those tumours having the clinical appearance of mycetomas but which are not caused by fungi.

Historical

H. V. Carter (1860) coined the term mycetoma to designate lesions of fungal origin within subcutaneous tissue characterized by tumefaction, fistulization and mycotic granulation in the pus. Brumpt (1905) defined mycetomas as follows: mycotic tumours formed by a filited mycelium and susceptible to external elimination through more or less well developed sinuses. Brumpt has established that these tumours could be caused by very different fungi. Pinos (1913) proposed a distinction between actinomycosis caused by *Actinomyces* and *Nocardia* species and the true mycetomas caused by fungi having as their only common characteristic the possession of hyphae wider than those of the Actinomycetes. Chalmers and Archibald (1916-18) put forward the term Maduromycosis to denote the true mycetomas. It is thus evident that the term maduromycosis does not embrace all mycetomas. These writers similarly proposed the terms para and pseudo mycetoma. Bouffard (1919) distinguished three forms of mycetoma on a clinical basis: suppurating (*suppurée*), sclerotic (*scléreuse*) and cystic (*kystique*). Langeron (1936) claimed the mycetomas in two groups: the first includes the actinomycosis or actinomycotic mycetomas whilst the second contains the maduromycosis.

As to the term Madura foot it was according to Carter proposed in 1846 by Colebrook to name foot mycetomas in the region of Madura.

Importance and Geographical Distribution

The Madura foot type of mycetoma has a world wide distribution. It is however found especially in tropical or sub tropical region.

The disease is very much more frequent in male than in female and particularly in persons of mature age.

Etiology

The mycetoma would appear to be a consequence of wounding, folk and by subcutaneous inoculation of a saprophytic fungus. The disease is met with precisely in individuals who walk bare footed and are exposed to traumatism. The inadequacy of any simple explanation is however pointed out by Langeron (1936) who wrote "One may well inquire why *in toto* the mycetomas are accidental diseases and are not much more frequent in view of the multiplicity of opportunities of chance inoculation by fungi. Undoubtedly there is an important factor concerned in the genesis of this disease which is still completely elusive."

Pathogenic Agents

The pathogen of maduromycoses comprise about thirty different species distributed amongst ten genera. The list which follows is probably incomplete. It differs from those found in other work chiefly in two ways.

- (i) The actinomyces isolated from cases of maduromycoses seem only to be represented by a non sporulating, arobic species which might be assigned to the genus *Nocardia*. *Actinomyces israeli* in particular has not appeared to have been isolated from Madura foot. On the other hand *Nocardia asteroides* seems to be met with only in nocardiosis already defined.
- (ii) The genus *Moraxspora* met with in all classification is supposed the known species having already been referred by Fournier to *M. chelonoidis* which is the acoenotic form of *Moraxspora apiciformis*.

The essential characters of the genera *Maduraella* and *Thielia* are briefly given as well as those of *M. chelonoidis* (Shaw 1931).

List of Fungi Isolated from Cases of Maduromycoses

I. ACTINOMYCETES

(Genus *Nocardia*)

1. *Nocardia brasiliensis* (Lindenb.) C. G. Lund and Chalmers 1931

Several distinct species in which the rich chalky filamentous and radiating firm colonies adhere to the culture medium. The cultures emit a earthy odour. The colonies are yellow or orange. The granules formed by these *Nocardia* are white or yellowish.

Syn. *Discomyces brasiliensis* Landels 1931

Nocardia indica Chalmers and Christy 1930

Locardina med. nra (Vincent) Blanchard 1898

Non acid fast. Colonies glabrous humid soft wrinkled cream coloured. Granules white or yellowish.

Syn. *Streptothrix medusae* Vincent 1894

det. *nomyc. mexicana* Boyd and Crutchfield 1921

det. *nomyc. mycetomae* Crec. 1910

Discomyces fragilis n. Parajad Sal. 1919

3. *Locardina pelletieri* (Laeran) Lunos 191

Non acid fast and with a f. blk. development of the colonies which are glabrous acuminate wrinkled and of mucilaginous consistency. Colour pink to red. The granules are red.

Syn. *Microascus pelletieri* Laeran 1906

Locardina fricana Piper and Pullinger 1917

Locardina grac Froese 1930

4. *Locardina paraguayensis* (Almida) Conant 1947

Non acid fast species with smooth soft colonies having a whitish centre with the remainder dark in colour. The granules are black.

Syn. det. *nomyc. paraguayensis* Almida 1940

II ASCOMYCETES

1. Genus *Alleicheria*

Alleicheria boydii Shear 1921

Starting with granules on Sabouraud's agar colonies 6 cm in diameter covered with tufted hyphae of waxy consistency appear in two weeks. The colonies become dryer and greyer upon ageing. The thin walled mycelial hyphae are branched septate 1 to 3 μ in diameter and often coreniform. The upright conidiophores terminate with a single oval or pyriform uncellular spore measuring 2.1 to 10.4 μ by 3.7 to 8.7 μ and attached by a flat facet.

Enmons (1944) showed that *Alleicheria boydii* is the anamorphic form of *Monosporium prosperum* Saccardo 1911.

Giffert and Redaelli (1950) have established the complicated synonymy of this fungus which is one of the most frequent causes of maduromycosis in the United States as follows—

1. *Sclerosporium apiospermum* Saccard 1913

Monosporium sclerotiale Ieper 1914

2. *Monosporium nigrum* Peyer 1914

3. *Monosporium apiospermum* var. *sclerotiale* Ieper 1914

4. *Sclerosporium* sp. f. *Magalhães* 1919

5. *Alexisium prosperum* n. Main 1921

6. *Cephalosporium boydii* Shear 1922

7. *Dendrostilbella boydii* Shear 1922

- 9 *Glenospora boydii* Pollacci and Nannizzi 1928
- 10 *Indiella americana* Delamare and Catti 1927
- 11 *Scedosporium magalhãesii* Froes 1930
- 12 *Uacrosporium magalhãesii* Dodge 193
- 13 *Glenospora uridobrunnea* Redvelli and Ciferri 194

B Genus *Aspergillus*

- 1 *Aspergillus bouffardi* Brumpt 1906
- 2 *Aspergillus chevalieri* Mangin 1909

C Genus *Sterigmatocystis*

- Sterigmatocystis nidulans* var *Nicolleti* Pinos 190

D Genus *Penicillium*

- Penicillium mycetomagenum* Mantelli and Negr 191

III FUNGI IMPERFECTI

A Genus *Phialophora*

- Phialophora jeanselmei* (Langeron) Fillion 194
- Syn *Torula jeanselmei* Langeron

B Genus *Madurella*

This genus is represented by imperfect fungi which produce black granules in human tissue and which at 37°C develop upon Sabouraud's medium colonies which remain sterile. The genus *Madurella* erected by Brumpt in 1903 has as typical species *Madurella mycetomi* (Laveran 1902) Brumpt 190. Microscopic examination of the colonies shows them to be made up of wide hyphae (1 to 10 μ) which develop chlamydospores. No other forms of reproduction have as yet been observed.

- 1 *Madurella mycetomi* (Laveran) Brumpt 190
- Madurella bovis* Brumpt 1910
- 3 *Madurella tomentosa* (Nicolle and Iltis) Pinos 191
- 4 *Madurella Oswaldi* Parmentier Horta 1919
- 5 *Madurella subarctica* Blum and Brun 1919
- 6 *Madurella ramiroi* Pirajá da Silva 1919
- 7 *Madurella americana* Cammel 192
- 8 *Madurella idahoensis* Cammel 19
- 9 *Madurella rifsum* Ca. tammaro 19
- 10 *Madurella Lockwoodii* Hanan and Jurek 1935

C Genus *Indiella*

The fungi belonging to this group have never been cultured. Their descriptions are thus very incomplete and the species described so far are based on granule characteristics only. The granules are white.

- 1 *Indiella manoni* Brumpt 190
- Indiella regnieri* Brumpt 190
- 3 *Indiella brumpti* Pirajá da Silva 19

D. Genus *Ctenospora*

- 1 *Ctenospora tharoumensis* Chalmers and Archibald 1916
- 2 *Ctenospora armon* Chalmers and Archibald 191
- 3 *Ctenospora claspersi* Montpellier-Catanus and Clasper 1917

F. Genus *Cephalosporium*

- 1 *Cephalosporium reciferi* Leao and Lobo 1934
- 2 *Cephalosporium* sp. Carrion 1940
- 3 *Cephalosporium* sp. *lousal* Weidman and Hightman 194

Note: Several attempts have been made to modify the systematics of the genera *Madurella* and *Indovella*. The work of Ciferri and Fedalelli (1941) and of de Vello (1947) should particularly be referred to.

These diverse fungi have one feature in common, namely the formation of characteristic granules in the parasitized tissues. These are of definite form, size and of variable colour. The *Nocardia* species produce granules of the same microscopic structure as those of *Actinomyces israeli* in the classical actinomycosis and conform to the description already given. These granules are white, yellowish, pink, red or black. The granules produced by fungi other than *Nocardia* are usually larger and are composed in the centre of a mass of segmented and branched hyphae which develop chlamydospores. Chlamydospores are often found at the periphery of the granules. Between the hyphae which make up the granule is pigmented amorphous matter which imparts the characteristic colour to the granule. The shape of the granule may be oval, round or vermiculate. Colour of the granule cannot be used to classify the mycetomas. However, as a broad generalization, white and yellow granules are produced by *Nocardia* or *Indovella* species or by *Allocheria boydii*, while black granules are produced by *Madurella* and red ones most frequently arise from *Nocardia pelletarii*.

Symptomatology

Most cases of maduromycosis are localized in the foot, more rarely localizations are found in the hand, leg, forearm and even in the shoulder. Typical Madura foot is a globe foot in which the normal plantar concavity has been replaced by a convex surface. Three features characterize the lesions: tumefaction, fistulization and granules. The first appearance is usually very slow and consists of a little papule or nodule which develops, softens at the base and at length opens to the exterior. The sinuses may be single at first, but they usually continue to multiply and the discharge is oily and contains the characteristic granules. The disease which in consequence of traumatic injection is at first confined to the subcutaneous tissue, penetrates into all the tissues of the foot, which it transforms into a mass of sclerotic tissue traversed by sinuses and sometimes cavities in which large masses of granules are to be found. Several

years (10 to 15) are usually required for the full development of the characteristic Madura foot. The diseased foot may enlarge to a considerable volume so as to be scarcely recognizable with however little sign of pain the patient walk on a globe of mass which serves as a support. His general condition remains satisfactory until such time as a secondary infection provides a complication.

Histopathology

Brumpt (1906) and Montpellier and Catana (1934) give masterly descriptions of Madura foot. According to the latter authors the centre of the primary nodule is occupied by a granule of the parasite which is thus surrounded by a nest of leucocytes the component of which all polymorphonuclear neutrophils some intact others pyknotic are disseminated in a thin fibrous stroma. This leucocyte centre is in contact with a second zone rich in fibroblastic cell and neocapillaries of an inflammatory type the opening of which are nearly filled with the tumefied epithelium. The inner region of this zone abounds in polymorphonuclear cell. Large round or oval element are also present measuring almost 40μ with rather precise contours. Their nuclei have the structural characteristics of epithelioid cell. Often thrown back and depressed at the periphery they are frequently indistinct. However bi and trinucleate element may be seen. The acidophilic protoplasm approximates to 1 irregularly spongy it contains up to ten polymorphonuclear element in various stages of lysis. The practically constant occurrence of these macrophages sometimes seen at the periphery of the central leucocyte magna impart a distinctive appearance to sections especially at low and medium magnifications. The fibroblast increase in number in proportion to their distance from the centre the tissue of the stroma at first finely reticulate becomes more dense with a fibrillar outline the polymorphonuclear element gives way to mononuclear especially of a plasmocytic type which are very abundant at the periphery. Discreet eosinophilic and plasma cells are rarely encountered. Pigmented cells of which the pigment gives ferric reaction swarm in the second zone apparently matching the fragility of the capillaries. The presence of giant cell is constant a feature of other mycoses has not been observed in this mycotic tumour.

However Brumpt and most other authorities on the presence of giant cells to be a constant feature.

Treatment

If the condition is due to a *Nocardia* infection may commence with penicillin or sulphamides or a combination thereof. These may be ineffective in the case of mycetomas caused by actinomycetes or *Exophiala* imperfect where an amputation sufficiently extensive to ensure the complete eradication of diseased tissue is necessary. Relapses are infrequent.

Prognosis

This is always bad if not good, at least so far as the diseased organ is concerned. Indeed so far as present knowledge goes amputation is almost always necessary.

Differential Diagnosis

Diagnosis of maduromycosis is so obvious that confusion with other diseases is likely only in the early stages. No erythema as in syphilis and tuberculosis may present the same clinical picture though no other disease yield the triad of tumefaction, fistulization and granules. Other mycoses (*blastomycosis* or *coccidioidomycosis*) elephantiasis, mycetozoa and *sporotrichosis lymphatica* may be at first recall the picture of maduromycosis.

Mycological Diagnosis

This consists simply of finding and culturing the granules and is important from the therapeutic as well as the theoretical point of view. The granules are found by the naked eye or by means of a lens then examined fresh or in potassium hydroxide. Very hard granules are softened in warm potassium hydroxide or boiling Eau de Javel. Before inoculation they are rinsed in sterile water.

Experimental Inoculation

The clinical picture of maduromycosis has never been reproduced from cultural isolates.

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CHAPTER VIII

White Piedra

THE WHITE PIEDRAS sometimes called trichosporia or *piedra andra* are diseases characterized by rather soft whitish or brownish nodules which develop upon the hairs of the moustache or beard most rarely upon the hair of the head or superfluous hair. The disease is cosmopolitan.

The irregular nodosities raise the cuticle of the hair and produce upon its surface a mosaic of vaguely quadrangular elements from 1 to 4 μ in diameter.

The fungus which causes white piedra is *Trichosporon beigeli* (Lal.) N. Bord. (Willemin 1902) for which there are several synonyms:

<i>Leucococcus beigeli</i> Rabenh. 1883	<i>Trichosporum orale</i> Unna 1879
<i>Sclerotium beigelinum</i> Haller 1884	<i>Trichosporum cerebriforme</i> (Ham.) Hayashi Ota 1924
<i>Zootecia beigeli</i> F. Werth 1887	<i>Trichosporum granulatum</i> (Ham.) Hayashi Ota 1924
<i>Hyalococcus beigeli</i> Schröter 1888	<i>Trichosporum humilimum</i> n. sp. Marz and Nino 1923
<i>Chlamydotomus beigeli</i> Trevisan 1889	<i>Piedra columbiana</i> Duxig 1871
<i>Trichosporum oroides</i> Behrend 1890	<i>Trichosporum minus</i> Lal. 1940
<i>Trichosporum gignileum</i> Behrend 1890	

Cream coloured colonies of *Trichosporon beigeli* grow easily upon Sabouraud's medium and have a membranous and folded appearance. Microscopical examination shows numerous filamentous arthrospores in which they resemble *Crotrichum* colonies and also appendages which are not found in *Crotrichum* colonies.

Nodules of white piedra may be distinguished by their colour from those of black piedra by their consistency being soft and by the absence of ascopores in the knotted regions of the mycelium.

The best treatment would appear to be to shave off the moustache beard or if this drastic procedure is inconvenient to apply dilute (1:1000) lotions twice daily.

Although white piedra has been known much longer than black piedra from which this has often been hardly distinguished only since 1911 the impression is that the former are much less well known than the latter and that there is need for purification of the nomenclature of the various species hitherto described. The rarity of the disease and its lack

of importance in human pathology tend to obscure studies of a primarily theoretical nature upon the causative agent.

Pedaell and Ciferri (1941) have studied various fresh strains of *Trichosporon* and regard this genus as having affinities with the asporogenous yeasts taking its place with the subfamily *Trichosporonoidae* (Nannfeldt). Ciferri and Pedaell (1936), Duddens and Lodder (1942) regard the *Trichosporon* as an anamorphous yeast-like fungi which with the *Candida* and *Brethomyces* species comprise the subfamily *Mycotorulaceae* characterized by the formation of a pseudomycelium. The subfamily *Torulopseudomycetaceae* in which no pseudomycelium is formed constitutes with the *Mycotorulaceae* the family *Torulopseudomycetaceae*. Langeron (1st edition of the *Precis de Mycologie* p. 44) criticizes these authors for having taken as their type *T. cutaneum* which is common saprophyte and not pathogenic *Trichosporon*.

Pedaell and Ciferri (loc. cit.) give a list of nine pathogenic species of *Trichosporon* for whose pathogenicity we have little respect as follows—

- 1 *Trichosporon asotii* (Mon. and Vassina) Cif. and Ped.
Trichosporon beigei (Rabenhorst) Willemsen
- 2 *Trichosporon Lechetsii* Ped. and Cif.
- 4 *Trichosporon cutaneum* (de Beurman and Cougerot) Ota
= *Hemyspora coremiformis* Moore
= *Trichosporon rugosum*
- Trichosporon giganteum* Behrend
- 6 *Trichosporon proteolyticum* Negroni and De Vill. Lastra
- 7 *Trichosporon Baloni* Ota
- 8 *Trichosporon granulorum* Ota
- 9 *Trichosporon affinale* Cif. Cron and Brun.

Langeron's words (1st ed. of the *Precis* p. 546) provide an appropriate conclusion. Finally the white pedras are produced by a group of arthrosporic fungi equipped with complicated apparatus comprising species very close to one another and perhaps identical differing particularly in their geographical distribution. Perhaps there is only one *Trichosporon* for white pedra?

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CHAPTER XIV

Black Piedra

THIS INFECTIO, characterized by hard black nodules along the length of the hair, is somewhat common in South America and has been recorded from Java and Cochun China. The fungus which causes it is *Piedraia hortii*.

The nodules may or may not be visible to the naked eye, but when the hair is combed they produce a gritty sensation. Several species of fungus have been held responsible for the infection but it seems likely that it is due to a single species, namely *Piedraia hortii* (Brumpt) Fonseca and Liao 1939. If infected hairs are examined in potash the nodules are seen to be below the cuticle and formed from thick mycelial filament with brownish or blackish walls reduced to arthrospores. Among the filaments are found fusiform ascospores with a filament terminating each pole. The structure of the mycelial filaments compares with that which Arnould named a cistroma. The asexual spores are apparently formed from one cell of the ascostroma which becomes oval or pear-shaped. The mechanism of liberation of the ascospores from the ascus is not yet known precisely but it is preceded by a gelatinization of the cell surrounding the ascus.

Inoculation of Sabouraud's agar with isolated ascospores or with nodules produces a black acuminately-flocculent glabrous colony composed of thick mycelial filament with short cells and many chlamydospores among which asexual and ascospores may sometimes be seen.

The following are synonyms of *Piedraia hortii*.

<i>Trichosporum hortii</i> Brumpt 1913	<i>Piedraia rosei</i> et al. Brumpt and Langeron 1934
<i>Trichosporum paraguayensis</i> Delamare and Catti 1928	<i>Piedraia roussei</i> et al. Lh. 1936
<i>Piedraia acuminata</i> Pereira 1930	<i>Piedraia jamaica</i> Baxby and Verhulst 1938

Diagnostic differentiation from trichomycosis is trichomycosis may be treated consist of rubbing twice daily with 1% (w/v) salicylic acid or the hair may be shaved off.

During his work on Arnould's thesis (1934) Langeron attributed to him (1939) that *Piedraia* is an Ascomycete fungus belonging to the order *Uromyces* order Microthrales and near to the family Microthrales. The *Ascomycetes* described by Arnould from a group of parasites adapted to superficial parasitism. They require a very humid

climate for their development and are only found in regions with high rainfall of a metre or more per year. Langeron who with Brumpt described *Piedraia rosea velensis* (1934) as having 4 spored aeci and equal ascospores finally believed (see 1st edition of *Précis de Mycologie* p. 547) that this was a borderline case of *P. horta* very closely related to this species and differing only in the form of the pedraie nodules, the number of ascospores per ascus and the length of the polar filaments.

The fundamental work on black piedra is that of Parreiras Horta (1911) which Brumpt acknowledged by designating *Trichosporium horta* (1913) which ultimately became *Piedraia horta* (O da Fonseca and A. M. de Lea, 1928).

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CHAPTER XIV

Black Piedra

THIS INFECTION characterized by hard black nodules along the length of the hair is somewhat common in South America and has been recorded from Java and Cochun China. The fungus which causes it is *Piedraia hortai*.

The nodules may or may not be visible to the naked eye but when the hair is combed they produce a gritty sensation. Several species of fungus have been held responsible for the infection but it seems likely that it is due to a single species namely *Piedraia hortai* (Brumpt) Fensholt and Læo 1928. If infected hairs are examined in potash the nodules are seen to be below the cuticle and formed from thick mycelial filaments with brownish or blackish walls reduced to arthrospores. Amongst these filaments are found fusiform ascospores with a filament terminating each pole. The structure of the mycelial filament compares with that which Arnaud named *ascostroma*. The asci are apparently formed from one cell of the *ascostroma* which becomes ovoid or pyriform. The mechanism of liberation of the ascospores from the ascus is not yet known precisely but it is preceded by a gelatinization of the cell surrounding the ascus.

Inoculation of Sabouraud's agar with isolated ascospores or with nodules produces a black acuminate folded glabrous colony composed of thick mycelial filaments with short cells and many chlamydospores among which asci and ascospores may sometimes be seen.

The following are synonyms of *Piedraia hortai*:

<i>Trichosporum hortii</i> Brumpt 1913	<i>Piedraia hortii</i> Brumpt and Langeron 1934
<i>Trichosporum paraguayense</i> Dalmari and Catti 1925	<i>Piedraia surinamensis</i> Dalmari 1925
<i>Piedraia surinamensis</i> Ieteira 1930	<i>Piedraia jurensis</i> Kuehny 1931 Verhulst 1934

Diagnosis. Differentiation from trichomycosis or trichoglyphosis may be made by treatment consisting of rubbing twice daily with 100% Fluimol or the hair may be shaved off.

Based on his work on Arnaud's thesis (1914) Langeron attempted to show (1929) that *Piedraia* is an Ascomycete fungus belonging to the class *Pyrenomyces* order *Microthyriales* and near to the family *Microthyriaceae*. The Ascomycetes as described by Arnaud form a group of parasites adapted to superficial parasitism. They require a very humid

CHAPTER VI

Pityriasis Versicolor

PTYRIASIS VERSICOLOR is a cutaneous infection characterized by irregular scaly spot—fawn to brownish in colour and spreading over the trunk. On examining the scales a large variety of mycelial element mixed with rounded forms are found the element have been named *U. hi. furf* (Robin) Bullon 1849 and are believed to be the cause of the infection.

Pityriasis versicolor has a number of synonyms—tinea versicolor, a brown purpura of the scalp, pityriasis versicolor tropica, tinea flavo tinea nigra, body spot, etc. The disease has a world wide distribution and exhibits a preference for young adult but also attacks the very young and the very old. In temperate climates it is usually confined to the trunk and facial lesions are exceptional. In tropical climates facial localization is very common (Vanbreuseghem 1940). The infection may also attack the limbs, the neck and the scalp.

The spots of pityriasis versicolor are of varied and variable colour—sometimes darker, sometime lighter than the surrounding skin. It is likely that the latter condition is due to protection of the skin from the sun by the scale. The outline of the spot is irregular, large or small and sometime minute. The scales are fine and scurfy. On examination in chloral hydropyrol which is preferable to caustic potash as a clearing agent rounded element are seen of diameter 1 to 4 μ after budding arranged in clusters and mixed with mycelial fragment 3 to 4 μ wide and 1 to 40 μ long sometimes branched. If the scales are stained before examination and not mounted in chloral hydropyrol the mycelium is seen to be a much branched system which is considerably broken up into fragments upon the addition of chloral hydropyrol or caustic potash.

Histological sections show that the filament and spores are in the superficial epidermal cell and that here and there they penetrate lightly into the dermis.

The causal agent of pityriasis versicolor has been named *U. hi. furf* (Robin) Bullon 1849. In the literature the following synonyms are still found e.g. *Microsporum furf* (Robin) 1849, *Malassezia* (Castellani 1905), *Malassezia tropica* (Castellani 1919). But the name only covers a morphological entity—the picture presented by the scales is always the same whether in a tropical or a European case (Lejune). It does not represent a biological entity because it is not possible to culture the agent of pityriasis versicolor when culture becomes possible several species may well be found and as in the case of *Microsporum* in

hair the complex clusters of spores and filaments may represent several botanically different species. A number of attempts to culture *M. furfur* have failed. However some workers, namely Iarj (1937) and Moore (1941) claim to have obtained cultures and the latter named *M. furfur* to inoculate an animal. Vanbreuseghem (1940) and Lajeune (1941) have



FIG. 44

P. furfur on skin. Small patches are visible on the back of a negro

made repeated attempts on a large scale. Vanbreuseghem tried unsuccessfully with the following media: Sabouraud medium, plain agar, Loewenstein medium, blood agar, Loeffler medium, Sabouraud medium with the addition of a fine layer of butter and direct inoculation of hairs *in vitro*. Lajeune has had no greater success; he repeated Moore's attempts and also tried the following media: Moore medium plus penicillin, Buller's medium, various media in an atmosphere of 10 per cent or 20 per cent CO_2 , media enriched with urea and others with vitamin B respectively.

On account of the failures it is impossible to speculate on the systematic position of the usual agent of pityriasis versicolor. Microscopical examination gives little indication of its position in the mycelium

CHAPTER XVI

Rhinosporidiosis

RHINOSPORIDIOSIS is a disease characterized by polypous masses formed on the mucous membranes more rarely on the skin and it is probably a mycosis. The causal agent has never been obtained in culture but has been named *Rhinosporidium seberi* (Wernicke) Beeber 1912. According to de Vello (1949) the number of published cases in human beings totals 44, distributed throughout the world as follows: Africa 1, N. America 18, S. America 34, Asia 377 (of which 108 are from Ceylon and 233 from India), Europe (Italy) 1, Philippines 1. Of the cases from Asia (which constitute 85.3 per cent of the total) 8.7 per cent were diagnosed in India and 4.4 per cent in Ceylon. According to de Vello the number of cases in animals is 70 of which 9 are from S. Africa, 11 from S. America and 50 from India. Thus rhinosporidiosis is primarily an Asiatic disease. Conant *et al.* (1947) reported the occurrence of rhinosporidiosis in Scotland and England, but the case reported by Ashworth to which they no doubt referred was of an Indian student at Edinburgh.

The disease affects young adult males. In India it is especially noticeable among those employed in lifting sand and gravel from river beds. It is possible that rhinosporidiosis is an infection of fish which will accidentally attack man and domestic animals. In an infected frog (*Hyla rubra*) Carini (1940) described *Dermosporidium hylarum*, a parasite resembling *Rhinosporidium seberi*.

The parasite described by Beeber in 1900 has the following synonyms—

<i>Coccidioides</i> sp. Wernicke 1900	<i>Rhinosporidium</i> sp. years Allen and Dave 1930
<i>Coccidium seberi</i> Wernicke 1900	
<i>Rhinosporidium</i> sp. I. analysis Minchin and Pantham 190	<i>Rhinosporidium</i> sp. not onium Abernethy 1944
<i>Rhinosporidium</i> sp. cf. Zschokke 1913	and probably <i>Dermosporidium hylarum</i> Carini 1940

It must be realized that the singularity of the causal agent of rhinosporidiosis based as it is solely on morphological consideration is not as conclusive as it would be if the parasite could be cultured. The phenomenon of convergence which is so common in parasitic fungi precludes the conclusive demarcation of a species on unique morphological features alone. Proof that this is so is given by the fumigoid forms which appear in the tissues after parasitization by several species of *Phialophora* responsible for chromoblastomycosis and also by the better known

example of the dermatophytes which have a great number of species but apparently only one morphological parasite with little variation.

All attempts to culture *R. seberi* and to inoculate man or animal have failed.

It has been stated that rhinopodisma is characterized by polypoid masses these may be sessile or pedunculated and in 50 per cent of the cases are developed on the nasal mucosa. The conjunctiva of the eye is also frequently infected. Polyps have also been recorded on the pharynx larynx urethra vagina sometimes on the skin and in one case in the brain.

The polyps are red crumbling masses which bleed easily. On their surface whit mark indicating the presence of the parasite can be recognized.

In its youngest stage the parasite is a round corpuscle greater in size than an erythrocyte (7 to 9 μ diam) and contains a nucleus and lipid reserve. It has a thin chitinous cell wall. This wall thickens and becomes double owing to the formation of a cellulose membrane and the cell attains a diameter of 30 to 60 μ when the nucleus divides. This nuclear division is repeated without lysis of the cytoplasm such cleavage only takes place when the corpuscle has attained a diameter of 150 μ and contains about 1000 nuclei. The parasite continues to live by until it has attained a diameter of 200 to 350 μ then it contracts through a pore in the original cell membrane. The spores are discharged by the sporangium () into the surrounding tissues or the natural world such as the nasal cavity or the conjunctival cavity where they enter the throats of neighbouring poringia. But it is preferable to carry out diagnosis by the microscopic examination of fragments of polyps crushed under a cover slip.

The polyps are produced as a reaction of the conjunctiva to animal or vegetable material. Infection of the conjunctiva is usually accompanied by more rarely by a polynuclear leucocytosis. Around the membrane of discharge poringia granules may be developed with giant cells.

Treatment of rhinopodisma consists of surgical removal. It follows by ketone solution. Various medicaments with local trivalent antimony have been applied without success. But antimony compounds (neodiboron) have proved useful in rhinopodisma in domestic animals produce similar symptoms to those found in man. The disease is known to attack horses mules and cattle.

The systematic position of *Rhizoglyphus seberi* and its relation to Ashworth (1933) tested the organism to be identical with *Rhizoglyphus* Ashworth (1939) places the parasite among Thymopodisma.

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CHAPTER XVII

Sporotrichosis

Definition

Sporotrichosis is a benign but chronic mycosis attacking the skin and subcutaneous tissues and forming ulcers which extend along the lymphatic ducts. The infection is caused by traumatic injection of *Sporotrichum schenckii*.

Historical

This may be divided into three very unequal periods during which the principles were discovered leading to our knowledge of this infection. Chronologically the 3 periods are—

- 1 The American period
- 2 The French period
- 3 The South African period

The first was a period of discovery, the second was of a descriptive nature and laid the foundation of our present knowledge, and the third was one of confirmation and integration of concepts and of the solving of new problems.

1 American Period In 1894 in the United States Schenck described the first case of sporotrichosis. Two years later Hethcote and Perkins discovered another case and gave the name *Sporotrichum schenckii*; the pathogen previously isolated by Schenck.

French Period This period commenced in 1897 with Dr. Burmann and Ramond's discovery of a case of sporotrichosis. M. Truchot and Ramond named the causal agent *Sporotrichum leucum* in 1901 and concluded that it differed from the American sporotrichosis which Dr. Burmann and Cougerot in 1906 had named *Sporotrichum schenckii* (Hethcote and Perkins 1900).

The initial stimulus to the study of sporotrichosis in France and elsewhere was given by the work of Dr. Burmann and Cougerot and especially by their publication of a very important monograph entitled *Le Sporotrichose* (1911). This noteworthy work emphasized on the one hand the diversity of the fungi responsible for sporotrichosis and on the other the serious nature of certain forms of this mycosis. The ideas just formulated were taken up again much later by Cougerot (1925) and as we think

resulted in misunderstanding of the nature of sporotrichosis in Europe and especially in France where it is a relatively frequent and serious disease and the nature of the same infection in other countries notably North America. It is probable that at the time when De Beurmann and Gougerot wrote a large number of unsuspected cases of sporotrichosis dealt with by mistake for other diseases had imparted a very grave clinical impression. However this does not cover the whole question. We believe at the time of writing that it is wrong to postulate the existence of one sporotrichosis specific to Europe and another confined to America.

3 South Africa Period This commenced in 1917 with the work of Iijsser and Pullinger who studied an outbreak of sporotrichosis among South African native miners. The period really began however in 1947 with the publication of a symposium entitled *Sporotrichosis* section in *Vires of the Ilthetrand* which reported observations made on 9000 cases of sporotrichosis diagnosed in both natives and Europeans in less than ten years. It did not contribute anything fundamentally new but it confirmed and extended what was already known and suggested that the infection is caused by a fungus that normally lives saprophytically on vegetable matter. It also raised interesting problems such as for example the parasite cycle within the host tissues.

It must be realized that this conception of the history of sporotrichosis in three periods is purely arbitrary.

Importance and Geographical Distribution

Sporotrichosis is a relatively rare though cosmopolitan disease. This is found to be especially true when it takes the form of an epidemic. The adult male is apparently most susceptible, no race is immune but contraction of the disease depends on individual resistance. Men of certain occupations are more likely to be infected than others. Small epidemics have been noted amongst florists. The sample already mentioned in South Africa shows us a vast epidemic that broke out among miners black and white alike. In France many cases were recorded during the early years according to De Beurmann and Gougerot seven years after the first observation of De Beurmann and Parnood more than 700 cases had been discovered. In actual fact according to our information the disease is rather rare and we have reason to believe that it is because the infection is not well known that recorded cases are few.

Etiology

The etiology of any mycosis is better known than that of *sporotrichosis*. *Sporotrichum schenckii* lives saprophytically on various plant materials living or dead. If a man or an animal receives a slight wound the organism enters through the skin.

The first important step of this demonstration was made by discovery of Gougerot (communicated by De Beurmann and Gougerot in 1909). He

discovered in the French Alps strain of *Sporotrichum* on the bark of a beech tree the leaves of a horsetail and dry grains of oat. These strains were indistinguishable from those of *S. burmanni*; their pathogenic power at first poor in the rat subsequently increases. Congratogalea a diagram (p. 14 *Le Sporotrichose*) of a *Sporotrichum* growing in an oat grain which was as perfectly developed in all microscopic characters as a culture produced in the laboratory. As a result several small epidemics were produced by handling plant and Foerster (1936) was able in 14 cases to infect Harberty by way of its spines. Benham and Heaton (1932) after having isolated non-pathogenic *Sporotrichum* from different flowers tried to infect carnation buds with *Sporotrichum schenckii*. The conclusion that may be drawn from this experiment is doubtful because even if the authors were able to re-isolate *Sporotrichum schenckii* from the decaying carnation bud as a result of the previous infection it does not necessarily follow that the pathogenicity of this fungus had been demonstrated for carnation for as Skinner Frimmon and Tsuchiya (1948) have pointed out it is generally accepted that *Sporotrichum schenckii* is able to develop on dead or decaying vegetable matter.

According to the South African workers (cf. Symposium) sporotrichosis always followed a slight skin wound. However of 92 cases recorded at Venterpost and at Consolidated Main Reef (441 and 124 cases respectively) only 1 per cent and 40 per cent respectively of the workers recall having been wounded. Nevertheless the workers most frequently attacked are those who are exposed to slight injuries during their work. Furthermore in experiment carried out on volunteers it has been demonstrated that *Sporotrichum schenckii* is capable of infecting the skin other than through an abrasion or when it has been irritated by hot dressings or severe rubbing even though the skin may not actually have been broken. In the mice it is on the wound untreated wound for propagation that *S. schenckii* develops. In practice this is the only important source of infection. *S. schenckii* has only once been isolated from the air and never from the ground or from water. The South African authors have also demonstrated that flies (*Musca domestica*) become infected after contact with patient bearing sporotrichosis lesions. They have shown that *S. schenckii* can be isolated from the feet and from the faeces of these flies. Also the fungus is able to live for several days within the body of the fly but it is generally accepted that in practice flies do not play an important part in transmission of sporotrichosis.

Pathogenic Agent

The causal agent of sporotrichosis is *Sporotrichum schenckii* (Hethcote and Perkins 1900) *S. burmanni* (Congratogalea 1900). In 1893 Matruchot and Hammond reported the existence of a new species isolated from the first European case by Dr. Burmann and Hammond and named *Sporotrichum burmanni*. The distinction into two species is based on the fact that the colonies developed by the parasite isolated by Schenck were white

while those isolated from the French case were black. Davis however (1913-1917) showed that little value could be attributed to a difference of pigmentation or to a difference in capacity to ferment sugars. Most authors agree that there is but one causal agent of sporotrichosis *S. schenckii*; and this agent exhibits certain variations in pigmentation and fermentative action. There is a considerable number of synonyms:

<i>Sporothrix schenckii</i> . Hethcote and Perkins 1900	<i>Sporotrichum carougeum</i> Langeron 1913
<i>Sporotrichum deaurum</i> Matruch and Raymond 1905	<i>Sporotrichum cancellum</i> Wolbach Simon and Meyer 1917
<i>Sporotrichum terodei</i> Splendore 1909	<i>Sporotrichum pseudopneumoniae</i> Benedek 1920
<i>Sporotrichum granulosum</i> Brumpt and Langeron 1910	<i>Sporotrichum epizooticum</i> (Brumpt) Achari 1920
	<i>Rhinocladium schenckii</i> Brumpt 1920

The Nature of the Parasite in Pus and Tissues

According to classical opinion research on the parasite in the pus or tissues of man remain for the most part non-existent. But even if such research is often of little value and does not constitute a sure diagnostic method it is incorrect to say as some workers do that it is impossible to detect the parasite in the pus by direct examination (Gougerot 1936). De Beurmann and Gougerot were the first to describe the tissue form of the fungus (1906) and Gougerot reconsidered the question in 1909. These tissue forms are described as boat or egg-shaped. The organisms are elongated bodies with rounded end about 2 to 3 μ wide and 3 to 5 μ long in which the protoplasm is concentrated at the two extremities. These bodies occur only rarely in men but are encountered abundantly in the pus of inoculated animals (rat). They are intracellular in the leucocytes or giant cell thus causing them to become erythritically packed.

A rarer form of *S. schenckii* both in man and animals is an asteroid form first described in 1904 by Splendore who discovered it in an infected woman in Brazil. It is this particular form which Splendore believed to be a new species and which he named *S. asteroides*. Tulice (1931) reported the same asteroid form in two infected people in Uruguay. Two more cases were discovered in North America by Moore and Ackerman (1948) and Pinkus and Crekin (1950). Asteroid forms have been seen by De Beurmann and Gougerot, Greco, Harter and Gruyer (1909) in a guinea-pig. Tulice described the asteroid forms as follows: Extracellular bodies surrounded by pyocytes irregularly rounded rather small the greatest diameter not exceeding 10 μ . Its structure is characteristic in the centre is a round corpuscle (spore) 2 μ in diameter the contents staining a diffuse blue-violet colour with haematoxylin and having distinct double wall which takes up a deeper violet coloration. The surface is covered with radially arranged protuberances of erythritically variable length (between 2 to 3

and \sim to 8μ). The projections spread in all directions. They seem to have the central body at varying levels and it is these structures which give the spores a teroid or sweet chestnut like appearance. These bodies take up eosin very strongly thus indicating their acidophile nature. Langeron (Taher 1931) regarded the form as simple *trichosporae*.

Splendore has shown by cultural method that these a teroid forms are capable of reproducing the fungus in its more usual form and that they constitute a stage in the parasitic state of *Sporotrichum*.

Until just recently a teroid forms were considered to be rare. However the observation of the South African workers justify a reconsideration of this view. They found a teroid forms in no less than 1 of the eyes on which they were working and starting with South African or North American strains reproduced them in animal. Their conception of the parasitic cycle of *S. schenckii* is as follows—

Stage 1—Represented by the boat like form or cigar bodies which resemble spores produced in culture by *S. schenckii*. Both spores and cigar bodies react identically to Gram staining, only slightly.

Stage 2—Certain boat like forms are transformed into cryptococcal element with a spherical form.

Stage 3—Represented by an increase in size of the cryptococcal to twice their original dimension.

Stage 4—The periphery of the bodies becomes covered with an eosinophilic coating.

Stage 5—Represented by the a teroid bodies with a central mass—the cryptococcal body—surrounded by delicate radiating eosinophilic projections which are easily destroyed during manipulation.

Asteroid bodies appear toward the sixth week after the initial natural or experimental inoculation. At this stage the a teroid and cryptococci are rare or lacking.

It is difficult to draw satisfactory conclusion. Are the observations of the South African workers due to biological differences in the strains which they have studied? Or have their observations been more critical because they have taken into account the extreme fragility of the strains upon which they worked? We cannot say for certain. Suffice it to say that their description of the a teroid bodies corresponds very closely with that given by Talice and the literature.

No mycelial elements are found in pus or tissue fragments of mycelium injected into tissue experimentally regardless of whether or not the spores appear to develop into boat form.

Macroscopic Nature of Cultures

On Sabouraud glucose agar at room temperature (20°C) colonies of *S. schenckii* appear on the third or fifth day after inoculation with spores. Their appearance is absolutely different from that of most other pathogenic fungi. The colonies are not leathery. They are

as small smooth white round plate like or moist and somewhat resembling yeast colonies. In certain strains the white pigmentation persists indefinitely but often after a few days it becomes brownish or black. It will be recalled that it was on the basis of this difference in pigmentation that *S. schenckii* and *S. lecanium* were separated. As they grow older the colonies become more moist and the surface becomes corrugated and often covered with wick like protrusions. The colonies are of an elastic consistency.

Davis about 1911 was able to produce the boat shaped bodies which characterize the tissue form of *S. schenckii* either by culturing it anaerobically or by including a piece of fresh sterile animal tissue and using blood or serum as the medium. More recently Campbell (1917) obtained the yeast like phase of the boat shaped phase by culturing *S. schenckii* at 37°C under anaerobic conditions on Francis medium (blood agglutinated by gelatin and cystine). On this medium colonies appear after 36 to 48 hours like small yellowish bacterial growths consisting of elongated agar like bodies which reproduce by budding from one of their extremities. These bodies show a Gram positive reaction. This form lasts for long time if the culture on Francis medium is kept at 37°C. If the agar like bodies are kept at 30°C on Sabouraud's medium they reproduce the mycelial form. The culture from the mycelial to the yeast like phase from cultures maintained for some time in the laboratory requires several transfers.

S. schenckii will ferment glucose, lactose, lactulose and maltose but not dextrin, mannitol or dulcitol. Its action on the following substances is variable: icthamine, lactose, inulin, starch and glycerine (Marroquin 1947).

Macroscopic Nature of Cultures

It is not proposed to refer again to the yeast like phase described above. The mycelial phase is made up of mycelial filament spores and rarely blastospores. The hyphae have a diameter of something under μ they are hyaline multicellular and branched. The spores are borne on branch filaments upon the surface of the medium either terminally or around the articulations of the hyphae. They are borne singly on sterigmata 1 to 2 μ in length and about 0.5 μ in width but at the extremities of the hyphae the spores are aggregated to form an oval mass about 1 μ in diameter. The spores may attain a length of 3 to 4 μ and a width of 1 to 3 μ they may be brownish but are never black. Once detached from their parent mycelium they reproduce by budding. It is actually extremely difficult to make out the relationship of the spores with the parent mycelium when examining part of a culture teased out on a slide. The best preparations are obtained in practice by culturing the fungus directly on the slide or more simply De Beurmann and Gougerot long ago realized by dropping a coverslip on the surface of the colony. The coverslip is raised at a convenient opportunity and the material adhering to it is flooded and stained.

It is important to remember the impossibility of diagnosis in the vast majority of cases where there is not access to histological method. There is a tendency to reject a diagnosis of mycosis though the histopathological picture is not conclusive in that the pathogen has not actually been seen in the tissues. The simplest method of diagnosis and which gives the best and most constant result is that of culturing the fungus.

Treatment

Sporotrichosis has a unique position amongst therapeutics. It is confined to one medication only, namely potassium iodide. Antibiotics such as streptomycin and penicillin have been applied without success.

Couérot prescribed 2 g for the first day, 4 g for the third and fourth days then 5 or 6 g per day as the dose to be taken daily before meals.

Conant *et al* gave 10 drops of a saturated solution of potassium iodide three times a day and increased each dose by 5 drops three times a day until it had reached 30 or 40 drops three times a day. The drops are administered before meals and diluted with milk or water. Intravenous injection of 1 g p.d. of sodium iodide may be applied in place of potassium iodide ingestion.

The South African authors who have treated a great number of cases in their country adopt the following method for the first two days at the hospital the patient receives 2 g (30 grains) of potassium iodide three times a day in a soup-spoonful of water (½ ounce). They consider that if the symptoms of iodine poisoning are to appear they will do so during this time. If the symptoms are slight the treatment is continued without interruption. If they are serious the treatment is stopped for 48 hours and then resumed. The size of the dose is generally maintained at the initial level but may be raised until the daily dose totals 120 grains (9 g).

The iodide treatment should be continued for 4 to 6 weeks after apparent recovery. The treatment is very effective.

It is not advisable to increase the lesion because this often leads to long-term ulceration. Spontaneous ulcers can be treated with iodine. In certain cases it is an advantage to use an auto or a stock serum on patients who are not sufficiently responsive to iodine.

According to the South African work is a contraindication of this treatment applied to the inoculation, however, in 3 effect a cure. Several inoculations are not however affected by this treatment.

Prognosis

It can be said of practically every case that prognosis is good. If spontaneous ulcers and naturally resistant individuals are known. The clinical forms and certain of the mucous membrane lesions have a good fit of function by this treatment.

Differential Diagnosis

The localized lymph node form is difficult to confuse with other. However the primary lesion must be distinguished from a tubercle.

t berenlose, ungetragene Ecthyma und Tularemia. Of other mycoses chromoblastomycosis, coccidioidomycosis, North and South American blastomycosis and trichophytr granulomas may be confused with sporotrichosis.

Mycological Diagnosis

Although theoretically there are three methods of mycological diagnosis, namely examination of the pus culture and animal inoculation, culture remains the most trustworthy and constant method.

(a) Examination of Pus

Pus is obtained either from a softened nodule or from the exudate of the primary lesion and is examined for the presence of boat-shaped and asteroid forms. In man the boat-shaped forms had always been considered rare and the asteroid forms exceptional until the observations of the South African workers.

The boat-shaped and asteroid forms can be examined directly on a slide under coverslip. For the boat-shaped forms Area-Lago and Coto (1940) recommended staining by the May-Grunwald-Giemsa procedure. Staining is carried out on smears of pus diluted in 10 to 1 volumes of sterile water; the staining time should not exceed 30 min. By this method the Brazilian workers have been able to recognize not only the boat-shaped forms but also the budding forms.

(b) Cultures

The cultures are grown on Sabouraud's medium at a temperature of 30°C. A few large drops of pus taken from an unopened nodule by a syringe are spread over the agar surface. After 3 to 5 days the characteristic colonies appear. Campbell (1941) was able to produce the boat-shaped forms on Francis medium at 37°C.

To facilitate a rapid diagnosis Cougerot has devised a contrivance for running the pus on a dry glass tube. This method consists in allowing a large drop of pus to run along the wall of the tube in the angle formed between the agar surface and the wall when inoculating the agar. These parasites develop on the glass through which it is easy to see and examine them on the second or third day (Cougerot 1936). For this purpose the tubes are fixed with Plastine on the stage of a microscope and examined under a powerful eyepiece and a low-powered objective.

The South African workers have found that most of the lesions give negative cultures after 400 grains (about 26 g) of potassium iodide have been taken.

(c) Experimental Inoculation

The best experimental animal is the male white rat. The pus or an emulsion of the culture is injected intraperitoneally. There is rapid development (in 1 week) of an orchitis and peritonitis characterized by

little white nodules spread throughout the mucosa and the peritoneum. Examination of the secretion reveals the presence of numerous boat shaped Cramp positive intracellular bodies.

Resuming the experimental study of sporotrichosis in the mouse Baker (194) showed that in this animal *S. schenckii* causes the development of a disease so chronic that it is usually fatal. In the tissues of the mouse are found vast numbers of boat shaped bodies.

Serological Diagnosis and Allergic Reactions

1. *Nidal and Abram:* Sero diagnosis. About 1909 Nidal and Abram applied an agglutination test to the diagnosis of sporotrichosis.

A spore suspension is prepared by removing fragments of a 4 to 6 weeks old culture. The fragments are ground up in a mortar diluted with sterile water and the suspension is then filtered. The filtrate is examined on a slide to ensure that it is rich in spores but contains no mycelial filament. The suspension is agglutinated by serum from patient infected with sporotrichosis for 15 to 60 minutes at dilutions ordinarily of the order of 1/100 to 1/400 but which may go as far as 1/800 and beyond. In fact the carrying out of this procedure is not so simple as may at first appear. It is difficult to obtain a homogeneous spore suspension and many cross reactions have been observed. This method is inferior to diagnosis by culture.

The same may be said of the complement fixation reaction.

2. The following reactions have also been studied and found to lack specificity: the cutaneous reactions (Bruno Bloch) the subcutaneous reactions (Pautrier and Lutembacher) the intradermal reaction (De Beurmann and Cougron).

Spontaneous Sporotrichosis in Animals

Sporotrichosis has been recognized in a large number of animal species including the rat dog cat rabbit horse and mule. Lutz and Spindler (1907) were the first to describe spontaneous sporotrichosis in the rat. In this animal the lesions are subcutaneous and articular the paws and tail being most often attacked and the infection here taking the form of tumefactions filled with cheese like pus. Visceral and generalized lesions have also been recorded.

In all the animals affected the tissue form of *S. schenckii* is frequently encountered and in much greater numbers than in man. It is believed that in the rat the disease is transmitted from one animal to another by biting but this requires confirmation. It seems rather unlikely from what we know of the biology of sporotrichosis in human beings.

Treatment of animal as of man involves the application of sporicidal moccles (Carraguan has demonstrated the great value of this therapeutic method in horses and mules (1909)).

3. Transmission of sporotrichosis from animal to man has been recorded (Carraguan 1909).

Prophylaxis

The course taken by a sporotrichosis epidemic and the experiences acquired by the South African authors on the subject have given valuable information on the essential facts.

As already stated sporotrichosis usually occurs as isolated cases or more rarely in the form of small epidemics amongst, for example florists. In this case after diagnosis therapeutic treatment is begun. No special precaution is taken to prevent transmission to other individuals and as far as is known no case of contagious spread amongst human beings has been described.

This is not the case when sporotrichosis attacks a large number of people and when ideal conditions for its spread are realized. The various epidemics in the South African mines (Witwatersrand) are believed to be rare examples but are so important that they merit special attention. From the admirable series of observations and experiments made by South African specialists it is known that spread of such an epidemic depends on the following factors—

1. A pathogenic agent (*Sporotrichum schenckii*).

A vegetable substratum for the development of the pathogenic agent: wood in the mines.

3. A living organism receptive to the parasite: man.

4. An abrasion or wound.

Each of these factors is considered individually in relation to the hygienic prevention of infection that may be taken as follows—

1. The Pathogen

Although a large number of other fungi have been isolated it is established that all cases of sporotrichosis cited in the mines of Witwatersrand were caused by the one pathogen *S. schenckii* (based on an identification made by Fournier who called the parasite *S. brasiliensis*). Of 2 000 samples from sporotrichosis victims 1 400 yielded *S. schenckii* in culture.

2. Wood in the Mines

S. schenckii in the mines of the Witwatersrand lives saprophytically upon the wooden props. Its development on these props depends on various conditions—

(a) The wood must be of a certain type. Different species of eucalyptus and an acacia widespread in South Africa (*Acacia mellommis*) known as the wattie and various pines are suitable for the development of *S. schenckii*. However the fungus will not grow on the Oregon Pine (*Pinus taeda*).

(b) The wood must not have been treated by chemicals but may have been subjected to different stages of seasoning (e.g. desiccation etc.) without affecting the multiplication of the fungus.

(c) The wood must be healthy. The formation of mould on the wood reduces the development of *S. schenckii* considerably for reasons not yet understood. Among such antagonistic species are included *Polyporus* sp., *Hydnium* sp., *Lentinus lepidus*, *Polyporus rugulosus*, *Poria* sp., *P. saporatus* and *Stereum apadicum*. Certain species such as *Coniophora cerebella* have no antagonistic action. The destruction of *S. schenckii* by these fungi takes several months but is usually almost complete after 4 months. It should be noted that if rotten wood is introduced it may become infected by *S. schenckii* but the development of the pathogen under such conditions is poor.

S. schenckii grows most easily on white wood from the heart of the tree. Its penetration into the wood never exceed 1 to 2 mm but in transverse section the organism may be found at certain points up to 7 cm deep. Thus though it has no great power of penetration it is able to gain ingress via any fissures which it may encounter.

The nature of the fungus varies according to the species of host wood but the colour of the colonies is usually grey, ochre or blackish. On some species of wood the colonies are viscous on others they are milk milk if lime being damp and greyish. When the wood is decayed the colonies have a woody appearance. It is impossible to recognize with certainty colonies of *S. schenckii* by macroscopic observation alone. The South African authors have said that the pores of this fungus are triangular the size of the triangle varying in length from 6 to 12 μ . By subjecting scrapings from a piece of wood to microscopic examination it is easy to recognize the triangular spores which the South African authors have a diagnostic value. If the strain isolated from the people were in fact the pathogen would be demonstrated by the inoculation of guinea pigs.

3. Man

The numerous cases of paronychia reported in native and European workers in the mines of the Witwatersrand and the prevalence of lungeria has provided ample evidence that the causative strains of *S. schenckii* developed on the people. A few cases of natural immunity have been recorded by inoculation which were not followed by infection.

4. Wounding

Until it has been apparent wounds were found to a limit of 1 cm. In a recent experiment it has been shown that an abrasion 2 mm in diameter and 1 mm deep is sufficient for the penetration of *S. schenckii*. It has been established that all the factors are necessary for infection. The first factor is the presence of the fungus and the second is the presence of a wound. The third factor is the presence of the fungus in the wound. The fourth factor is the presence of the fungus in the wound. The fifth factor is the presence of the fungus in the wound. The sixth factor is the presence of the fungus in the wound. The seventh factor is the presence of the fungus in the wound. The eighth factor is the presence of the fungus in the wound. The ninth factor is the presence of the fungus in the wound. The tenth factor is the presence of the fungus in the wound.

wooden props—which would be the most profitable to tackle! The introduction of the fungus into the mines could not be checked because it is not known how it is introduced though it may possibly come from infected individuals. It has indeed been found possible to contaminate wood with human pus. Once the wood is contaminated natural vectors other than man (e.g. water insects) suffice to transmit the fungus from contaminated to new wood. It seems certain on the other hand that it was not the new wood which introduced the fungus into the mine because wood has never been found to be contaminated before it enters the mine. Infestation of the wood by the pathogen can be controlled directly by spraying contaminated wood with fungicides or indirectly by treating the wood with fungicides before it is introduced into the mines or after it has been introduced but is still uncontaminated. Both methods have been successfully employed.

Impregnation of the wood is effected by spraying with a mixture (1st Mixture) containing 3 per cent zinc sulphate and 0.3 per cent Thiolith (product composed of sodium fluoride, dimutrophenol and potassium dichromate).

Spraying with Domicide (containing sodium pentachlorophenyl) and with pentachlorophenol was also effective.

By dealing to use only previously treated wood the South Africans have been able to control epulism successfully and quickly.

Taxonomy

In this chapter the binomial *Sporotrichum* has been accepted to designate the pathogenic agent peculiar to sporotrichosis. According to Vuillemin however the genus *Sporotrichum* adopted by Vitrubot for the pathogen discovered by Dr. Beermann and Lamond (1936) does not describe the pathogen of sporotrichosis which should be placed in the genus *Rhizoctonia*. This view was taken by Brumpt. In 1907 Laneyron wrote: "There are indeed two parallel series of sporotrichs one of them is in the Mucedinaceae and in part comprises the genus *Sporotrichum* Link 1909 with diffuse mycelium no differentiated conidiophores the conidia being borne directly on the mycelium or more rarely on small sterigmata and in part the genus *Rhizoctonia* Cord 1877 which differs from the former by the possession of distinct septate conidiophores at the end of which are projecting teeth bearing the conidia. The other series in the Dematiaceae comprises the two genera *Trichosporium* Fries 1849 and *Rhizoctonia* Saccardo and Marchal 1888. *Trichosporium* correspond to *Sporotrichum* with diffuse mycelium conidia usually sessile and borne irregularly along the filament. *Phaeoascus* is homologous with *Rhizoctonia* differing in that it has no differentiated conidiophores nevertheless it has the characteristic teeth and the dusky appearance."

It would appear to be difficult to assign to the genus *Rhizoctonia* a parasite such as *S. schenckii* which is not an authentic member of the



